ABSTRACTS

RITA

2015

Rabies in the Americas
Fort Collins, Colorado

HOW TO USE THIS BOOK

Abstracts are arranged in alphanumeric order based on the OP/PP (oral presentation/poster presentation) numbers displayed in the RITA26 Program. If you're looking for a specific abstract, please search on the OP/PP number.

CÓMO USAR ESTE LIBRO

Los resúmenes están dispuestos en orden alfanumérico basado en los números que aparecen en el Programa RITA26 OP/PP (presentación oral/presentación del cartel). Si usted está buscando un resumen específico, por favor buscar en el número OP/PP.

COMO USAR ESTE LIVRO

Os resumos são dispostos em ordem alfanumérica com base no OP/PP (apresentação oral/poster) números exibidos no Programa RITA26. Se você está procurando um resumo específico, por favor, procure no número OP/PP.

Name: Samantha Wisely

OP01

Email: wisely@ufl.edu

Session: Keynote Speaker

Country: United States

Title: The interface between community ecology and genetics in understanding rabies dynamics and approaches to successful control in meso-carnivores

Authors: Samantha M Wisely

Affiliations: Department of Wildlife Ecology and Conservation, University of Florida

Abstract: Controlling rabies in meso-carnivore reservoirs is a challenge that requires a multi-pronged approach to both understanding the disease system and using that knowledge to curtail transmission. Nearly all successful control programs target reservoir populations with vaccination campaigns that limit transmission among individuals. Yet, these vaccination campaigns are costly and demand that a sufficient proportion of the reservoir population acquire immunity. Thus, successful control campaigns require a priori knowledge of wildlife ecology specific to the reservoir species targeted. Molecular approaches can enhance our understanding of reservoir behavior, population dynamics, dispersal and movement patterns which are essential to designing control programs. I present several case studies involving raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*), and Arctic foxes (*Alopex lagopus*) which use molecular approaches to understand the ecology of these rabies reservoirs. Particularly in the case of raccoons, these findings have helped shape control programs across the continent. I conclude with promising new molecular approaches that may aid in the control of rabies in meso-carnivores.

Name: Xiaoyue Ma

OP02

Email: hjv4@cdc.gov

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: United States

Title: Evaluation of a novel serological test for detecting rabies virus antibodies in bats

Authors: Xiaoyue Ma[1], Amy Turmelle Gilbert[2], James Ellison[1], Todd Smith[1], Subbian Satheshkumar Panayampalli[1], Darin Carroll[1]

Affiliations: [1] Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, Atlanta, Georgia, USA; [2] US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA

Abstract: Bats are the natural reservoirs for the majority of lyssaviruses globally. Bat-associated rabies is frequently reported and now constitutes a recognized public health threat in the Americas. In the United States, the major source of human rabies is from bats and spillover infection of rabies virus from bats to terrestrial mammals repeatedly occurs and leads to several rabies outbreaks. Numerous surveillances including sero-surveillance have been conducted to monitor the spread of rabies infection in bat populations. Micro-RFFIT, a serological method modified from the rapid fluorescent focus inhibition test (RFFIT), is currently used to detect rabies virus neutralizing antibody in bat serum. However, the disadvantage of time-consuming, labor-intensive, low-throughput and undetectable levels of rabies virus antibody in bats using current method limits the efforts of extensive bat rabies sero-surveillance. This study was designed to evaluate a new ELISA-based method for determining rabies virus antibodies in bats. This new technique utilizes electro-chemi-luminescence (ECL) detection to detect binding events and forms a unique solution offering a high sensitivity, dynamic range and investigator convenience. In this comparative study, RFFIT, micro-RFFIT, and the new ECL technique were used to evaluate the level of rabies virus antibodies in bat serum samples collected in North, Central and South Americas. The new ECL techniques demonstrated a sensitivity of 100% and a specificity of 98% for detecting rabies virus antibodies in bat serum samples. The preliminary results from this study appear promising and demonstrate the advantage of measuring rabies virus binding antibodies. This novel serological test offers a sensitive alternate method for sero-prevalence studies and enables a better investigation of antibodies development following rabies virus infection in bats.

Name: Stephanie Gatrell

OP03

Email: sgatrell@vet.k-state.edu

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: United States

Title: The effect of temperature and duration of sample storage on rabies neutralizing antibody stability

Authors: Sami Pralle, Stephanie Gatrell, Susan Moore

Affiliations: Kansas State University Rabies Laboratory, Manhattan, Kansas, USA

Abstract: Background and Purpose: Many of our collaborators involved in clinical and research trials are interested in long-term sample storage and often question the best method and the duration of long-term storage, ensuring accurate results. However, the effect of duration and storage conditions on rabies neutralizing antibody stability has not been extensively investigated. Therefore, the purpose of this experiment was to assess the stability of rabies neutralizing antibodies in serum and buffered diluent stored at 4°C and -80°C for various time periods. Method: Human and animal serum samples were initially analyzed for rabies neutralizing antibodies using the rapid fluorescent foci inhibition test (RFFIT). After analysis, samples were stored at either 4°C or -80°C for periods up to six years. Frozen samples were thawed, and all samples were analyzed at a second time point. Stability was assessed by confirming the results were within the acceptable variation of the assay of +/- two-fold of the initial reported result. Results: Most samples were stable for at least one year at all temperatures investigated, with many falling well within the two-fold acceptance range from the initial testing result. Human samples (100%) remained stable for one year when stored at either 4°C or -80°C with an additional heat inactivation after storage and before re-testing. Additionally, human sera samples stored in diluent, clear from visible contamination, remained stable when stored at 4°C for up to six years. Animal sample stability at 4°C was high at one year (88%) and declined at two (82%) and three years (71%). Conclusions: Analysis of rabies neutralizing antibody can be safely performed on uncontaminated samples stored properly at 4°C and -80°C for at least one year without any negative effects.

Name: Daria Manalo

OP04

Email: darsky1566@gmail.com

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: Philippines

Title: Production of rabies polyclonal antibody as reagent for the Direct Rapid Immunohistochemical Test (DRIT)

Authors: DL Manalo[1], MJC Ang[2], B Jarilla[1], E Avenido[1], L Tuason[1], C Demetria[1], A Estacio[2], BP Quiambao[1], LP Acosta[1], D Bobadilla, A Noguchi[3], B Bazartseren[3], N Suguira[3], Y Kaku[3], S Inoue[3]

Affiliations: [1] Research Institute for Tropical Medicine, Philippines; [2] College of Veterinary Medicine, University of the Philippines; [3] National Institute for Infectious Disease, Tokyo, Japan

Abstract: Introduction - Laboratory confirmation of rabies is essential for countries desiring to eliminate rabies. The most commonly used, and considered as gold standard for rabies diagnosis, is the Fluorescent Antibody Test (FAT). However, this test requires expensive equipment. There is a need to develop new rabies diagnostic test which is simple and can easily be used in the field. The Centers for Disease Control and Prevention (CDC) developed a new diagnostic method, the Direct Rapid Immunohistochemical Test (DRIT) which is cheaper, simple and rapid, which can be an alternative to FAT. It employs immunohistochemical test to detect rabies virus by incorporating various components of existing immunoperoxidase techniques. However, the reagent for this test is not commercially available, thus the Research Institute for Tropical Medicine (RITM) developed anti-rabies polyclonal antibody and evaluated the reagent using the DRIT. Moreover, two fixations namely 10% buffered formalin and cold acetone of the brain touch impressions were compared. Methodology - Rabbit was immunized and given booster after 3 weeks and every week thereafter for 6 boosters using rabies N plasmid DNA (RABV-N cDNA) antigen (from the National Institute for Infectious Disease, Tokyo, Japan) by shimadjet injection. The antibody was monitored weekly by indirect fluorescent antibody test (IFAT). Following a final injection, the rabbit was bled out and serum was collected. Rabies polyclonal antibody was purified from the serum using the MAb Trap Kit and biotinylated with NHS-LC-Biotin. Brain tissues from dog's heads submitted to RITM rabies laboratory and examined for rabies antigen using FAT were retrieved. Touched impressions were done on these brain tissues and separately fixed in 10% formalin and cold acetone. DRIT evaluation was performed on these fixed brain impressions. Result - Biotinylated rabies polyclonal antibody as reagent for DRIT was produced in RITM. A total of 142 samples were tested and 104 samples were positive both for FAT and DRIT; and 38 were negative. The diagnostic performance of DRIT with acetone and formalin fixation is comparable with dFAT. There were no false positive and negative results for both the acetone and formalin fixation of DRIT. The sensitivity, specificity, positive and negative predictive values were all 100%. There was no difference between acetone and formalin fixation. Conclusion and recommendation - The DRIT can be an alternative diagnostic test for rabies antigen detection in areas where dFAT is not available. Moreover, dFAT can be a confirmatory test for DRIT or if dFAT result is ambiguous, DRIT can be utilized to verify the result. Further evaluation using bigger sample size and utilizing brain tissues samples from all over the Philippines is needed to fully recommend the use of DRIT.

Name: Yu Li

OP05

Email: yuli@cdc.gov

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: United States

Title: Development of quantitative reverse transcription PCR assays for the diagnosis of rabies

Authors: Yu Li, Ashutosh Wadhwa, Kimberly Wilkins, Jinxin Gao, Rene Edgar Condori, Andres Velasco-Villa, Darin Carroll

Affiliations: Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, Atlanta, Georgia, USA

Abstract: Rabies, caused by rabies virus (RABV), is one of the most dreaded zoonotic diseases, with a nearly 100% case fatality rate. Although preventable, at least 55,000 people die each year from rabies worldwide. Rapid and accurate laboratory diagnosis of rabies in humans and animals is essential for timely administration of post exposure prophylaxis. Currently, the direct fluorescent antibody test is the only recommended diagnostic method for routine RABV determination. However, reverse transcription-PCR (RT-PCR) has emerged as an alternative sensitive and specific method for rapid RABV diagnostics. Here, we present the validation of a newly developed pan-rabies quantitative RT-PCR (qRT-PCR) assays using RABV variant panels and clinical samples. Primers and probes of the new assays were designed based on alignments of over 200 genomes of RABV and lyssaviruses published in the NCBI GenBank. The primers and probes were selected based on highly conserved sequences to achieve the sensitivity and specificity necessary to detect a broad range of RABV variants and lyssaviruses. The new assays were validated using a selected panel of over 80 RABV variants in the CDC RABV depository and other rabies/lyssavirus panels. Serial dilutions of RABV RNA showed that the new assays were consistently able to detect RABV RNA at 10-6 dilution of clinical samples. Two qRT-PCR assays, N34 and N56, were able to detect all the positive RABV samples. The new rabies qPCR-PCR assays have successfully tested over 110 suspected rabies specimens at CDC during the last 7 months without false positives or false negatives. By utilizing artificial positive controls in conjunction with a beta-actin qRT-PCR assay as a negative control, the new rabies qRT-PCR assays provide rapid, sensitive and robust tests for RABV RNA and enhance laboratory rabies diagnostic capacities.

Name: Subbian Satheshkumar Panayampalli

OP06

Email: xdv3@cdc.gov

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: United States

Title: Mass spectrometry based diagnosis of rabies virus infection in clinical samples

Authors: Matthew S Reed, Olga Stuchlik, William C Carson, Darin Carroll, Yu Li, Jan Pohl, PS Satheshkumar

Affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Abstract: Rabies virus (RABV), a prototype member of the genus lyssavirus has negative-sense RNA genome. It is a highly neurotropic virus that infects human and animals causing a fatal encephalomyelitis. Rabies accounts for more than 50,000 human deaths annually, the majority in Africa and Asia where canine rabies remains endemic and availability of biologics for post-exposure prophylaxis is limited. Immediate post-exposure prophylactic treatment with antibodies of infected individuals is important to prevent transmission of rabies virus from peripheral tissues to brain. Thus, there is considerable importance for accurate and rapid identification of rabies virus infections. Rabies virus diagnosis relies on several tests that includes detection of antigens (DFA, dRIT), antibodies (RFFIT, IFA) and nucleic acids (RT-PCR). Since, spread of RABV to central nervous system is more critical for disease transmission, detection of antigens and nucleic acids in brain are more important than antibodies. Antigen detection (predominantly against RABV Nucleoprotein [N] protein) depends on microscopic detection, either based on fluorescence (DFA) or histochemistry (dRIT) with anti-N protein antibodies. DFA (direct fluorescent antibody) is the predominant diagnostic test used for rabies antigen detection in brain and skin samples. Although, DFA is very effective in identifying positive samples based on intensity and pattern of fluorescent staining, it has major drawback due to non-specific and cross-reactive staining patterns (in <1% of clinical samples). The nature of the tissue sample is critical for DFA analysis, since disintegrated or contaminated tissues cause antibody staining patterns difficult to make positive interpretation. These indeterminate samples need additional testing either by RT-PCR or electron microscopy. RT-PCR is highly sensitive and specific assay, but depends on the stability of RNA in tissue samples for amplification. Proteins / antigens are more stable and could be detected using alternate techniques. In this study, mass spectrometry based techniques was employed for detection of rabies virus encoded nucleoprotein in tissue samples. The results and potential advantages of this novel technology for rabies detection will be discussed.

Name: Rodney Willoughby

OP07

Email: rewillou@mcw.edu

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: United States

Title: Cerebrospinal fluid metabolomics rapidly discriminates rabies from other inflammatory brain diseases

Authors: Caitlin D French[1], Rodney E Willoughby[2], Esther D Mwaikambo[3], Donald L Granger[4], John F Foley[4], Susan J Wong[5], Darya O Mishchuk[1], Carolyn Slupsky[1]

Affiliations: [1] University of California at Davis, USA; [2] Medical College of Wisconsin, USA; [3] Hubert Kairuki Memorial University, Tanzania; [4] University of Utah, USA; [5] New York State Department of Health, USA

Abstract: Rabies encephalitis is rapidly progressive and usually fatal. Many mimics challenge diagnosis. Decisions on treatment are emergencies. Seven diagnostic methods are required to achieve 90% sensitivity with high specificity. Many countries lack rabies reference laboratories or adequate transportation infrastructure, limiting diagnosis and treatment of rabies. General platforms such as mass spectrometry and nuclear magnetic resonance (NMR) are far more ubiquitous, even in low-income nations, than rabies reference laboratories. Data files are easily analyzed by internet. We tested whether NMR metabolomics could differentiate between known inflammatory brain diseases. METHODS: Anonymized, residual CSF obtained from patients with proven rabies, West Nile or Lyme meningoencephalitis, cerebral malaria, multiple sclerosis and controls were tested. NMR spectra were obtained using 500 ul of CSF, filtered with addition of DSSd6 internal standard, on a BrukerAvance 600-MHz instrument at 10 minutes per sample. We identified metabolites using Chenomx NMR Suite v6.1 (Edmonton, AB, CA). Patients were grouped by PLS-DA using SIMCA (Umetrics, San Jose, CA, USA). Significant differences in metabolite concentrations were determined by Kruskal-Wallis ANOVA (RStudio 0.99.451, Boston, MA, USA) RESULTS: Rabies patients (n=6) clustered tightly and clearly separated from other diseases by metabolites related to energy metabolism, neural activity and oxidative stress. Multiple sclerosis and controls were most similar. High inter-individual variation was seen among West Nile (n=5) and malaria patients (n=3). DISCUSSION: NMR metabolomics may be useful for rapid screening of encephalitis patients, and is broadly accessible. As for any screening test, diagnosis of rabies will require confirmatory testing. NMR metabolomics may also be useful for timely therapeutic monitoring or to determine the futility of further medical care. Further validation of this approach with an expanded set of disease samples, under field conditions, is required. To this end, evaluation of NMR metabolomics for encephalomyelitis in children in Milwaukee, WI, USA is ongoing.

Name: Aaron Anderson

OP08

Email: aaron.m.anderson@aphis.usda.gov

Session: Economics of Rabies Control

Country: United States

Title: BioEcon: An agent-based stochastic simulation model for wildlife population and disease management with an application to canine rabies

Authors: Aaron Anderson, Brody Hatch, Chris Slootmaker, Stephanie Shwiff

Affiliations: US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA

Abstract: We present an agent-based stochastic simulation model (BioEcon) of wildlife population and disease dynamics under different management strategies. BioEcon can be parameterized for many different wildlife species and diseases, and it can accommodate lethal removal, fertility control, and vaccination efforts that vary spatially, temporally, and demographically. BioEcon allows managers to search for strategies that minimize costs subject to achieving target abundance or disease prevalence. Alternatively, the model can be used to search for strategies that maximize biological impact subject to some budget constraint. Other potential uses include estimating the benefits of longer-lasting contraceptives or vaccines, the benefits of more effective trapping, and the additional funding required to substitute fertility control for lethal management. We demonstrate application of the model by examining the impacts of alternative strategies for managing rabies in free-ranging dogs.

Name: Charisma Atkins

OP09

Email: gyq5@cdc.gov

Session: Economics of Rabies Control

Country: United States

Title: An applied example of RabiesEcon: a spreadsheet-based tool for evaluating the economics of rabies control programs

Authors: Charisma Atkins, Jesse Blanton, Martin Meltzer

Affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Abstract: To provide public health officials with analyses of alternative control programs for dog rabies, which results in nearly 60,000 deaths/year globally, we built a spreadsheet-based modeling tool, RabiesEcon. We used data from the Philippines to calculate the dog and human rabies cases averted by annual and semi-annual dog vaccination programs. For these programs, the percentage of dogs vaccinated was assumed to be 35% and 20% (biannually), respectively. We assumed two different human urban populations, using published census data, of 1.7 million in the capital city of Manila and ~40,000 in the city of Palayan, with dog densities of 8 and 4 humans per dog. We estimated the average cost per vaccinated dog to be U.S. $1.71 and post-exposure prophylaxis (PEP) to be approximately US$255/person fully treated. We used a 10 year time frame. A no vaccination program resulted in 179,132 dog and 252 human deaths over 10 years. Annual and bi-annual (twice yearly) 10 year programs cumulatively cost $3.9 Million and $5 Million, respectively, and averted 62,785 and 164,533 rabid dogs. An annual program resulted in a 3 year increase, compared to no vaccination, in dog rabies cases and an increase in human deaths. The bi-annual program prevents 217 human deaths. After 10 years, the bi-annual program nearly eliminated canine rabies; without continuous vaccination, however, the cases will again rise. The other programs resulted in a continuation of dog rabies over the 10 years and beyond. We found no notable differences between large and small urban areas in patterns of dog rabies. RabiesEcon, by allowing a user to readily alter input values and assumptions, can aid public health officials in designing cost-effective dog rabies control programs.

Name: Brody Hatch

OP10

Email: brody.g.hatch@aphis.usda.gov

Session: Economics of Rabies Control

Country: United States

Title: Toward canine rabies elimination: a comparison of three project sites

Authors: Brody Hatch[1], Stephanie Shwiff[1], Louise Taylor[2]

Affiliations: [1] US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA; [2] Global Alliance for Rabies Control, United Kingdom

Abstract: In excess of 50,000 people die annually from exposure to rabies, keeping rabies on the forefront of neglected diseases. Most deaths stem from being bitten by a rabid dog and while rabies remains a problem, those exposed must quickly receive post-exposure prophylaxis (PEP) or risk death. However, vaccination of dogs to control and eliminate canine rabies at the source has been implemented in many places around the world. Here we analyze three such case studies to determine possible factors in cost discrepancies.

Name: Louise Taylor

OP11

Email: louise.taylor@rabiesalliance.org

Session: Economics of Rabies Control

Country: United Kingdom

Title: Reassessment of the burden of canine rabies and its value to advocacy efforts

Authors: Louise Taylor[1], Katie Hampson[2], Louis Nel[3]

Affiliations: [1] Global Alliance for Rabies Control, United Kingdom; [2] University of Glasgow, United Kingdom; [3] Global Alliance for Rabies Control, South Africa

Abstract: Surveillance for human and animal cases of rabies is inadequate and therefore direct measurement of the burden of canine rabies is impossible at this time. Epidemiological modeling, based on the limited available data remains the only option. A recent extensive reassessment of the burden of canine rabies used the best available data to estimate the impact of canine rabies in individual endemic countries and globally. Outputs from a probability decision-tree model included the number of human deaths, the proportion of dogs vaccinated, the number of PEP treatments delivered, the costs of vaccination interventions in both canine and human populations, the value of livestock losses due to canine rabies and the burden of lost productivity due to human deaths. These primary outputs and analyses based upon them are valuable to support global advocacy efforts to increase investment in rabies control, and to help answer questions about where and how to focus investment in canine rabies control. They demonstrate (i) that through neglect, an entirely preventable disease continues to exert a heavy toll on the developing world, (ii) that surveillance efforts need to be dramatically improved to enable progress towards rabies control and elimination target to be assessed, and (iii) that investment in canine vaccination has a more profound impact on reducing human deaths than human vaccination alone can achieve.

Name: Todd Smith

OP12

Email: ire2@cdc.gov

Session: Human Rabies and Prophylaxis

Country: United States

Title: Ranpirnase inhibits rabies virus replication in vitro

Authors: Todd G Smith[1], Jamie Sulley[2], Thomas Hodge[2], Darin Carroll[1], Victoria Olson[1]

Affiliations: [1] Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, Atlanta, Georgia, USA; [2] TamirBio, San Diego, California, USA

Abstract: Currently, no rabies virus specific antiviral drugs are licensed or available. Ranpirnase is a 14 kDa RNaseA enzyme found in the oocyte of the leopard frog Lithobates pipiens (formerly Rana pipiens). Ranpirnase has been studied extensively for selective cytotoxicity in transformed cells and has been administered to more than 1,000 patients in a phase II/III clinical trial for mesothelioma. Ranpirnase is currently being repurposed and is under investigation for broad spectrum antiviral activity both in vitro and in vivo. Ranpirnase was added to cell monolayers 24 h pre-infection and remained in the medium for sampling 24, 48, and 72 h post-infection. Cytotoxicity was determined at these same time points using the neutral red uptake assay. Mouse neuroblastoma cells, BSR a clone of baby hamster kidney cells, and primary fibroblast cells (Eptesicus fuscus) were infected with fixed rabies virus ERA strain. Ranpirnase inhibited rabies virus replication in all three cell lines at all three time points. The 50% inhibitory concentration was in the low nanomolar range, and the 50% selective index ranged from 25 to greater than 800. Additional testing will further elucidate the antiviral activity of Ranpirnase against rabies virus. If proven effective in vitro, then crucial delivery experiments will be completed in vivo. The lack of a critical care animal model for rabies treatment is a major obstacle, and concerns about delivery of a large protein through the blood brain barrier remain. Despite these disadvantages, contingent on results of additional testing, Ranpirnase could be made available under compassionate use in the future.

Name: Rodney Willoughby

OP13

Email: rewillou@mcw.edu

Session: Human Rabies and Prophylaxis

Country: United States

Title: Rabies outbreak involving four transplant recipients in Kuwait and Saudi Arabia

Authors: Hussien Elsiesy[1,2], Iqbal Hussain[3], Faisal A Abaalkhail[1,2], Waleed K Al-Hamoudi[1,4], Mohamed I Al Sebayel[1], Dieter C Broering[1], Ahmed A Elgohary[1], Baderaldeen Ahmed[1], Sarah Al Fawaz[3], Sahar I Althawadi[3], Jawaher M Alotaibi[3], Hindi Al-Hindi[3], Prasad M Nair[5], Torki Al-Otaibi[5], Narayayanan MR Nampoory[5], Lillian Orciari[6], Andres Velasco-Villa[6], Pamela Yager[6], Todd Smith[6], Will Carson[6]; Mike Niezgoda[6], Ashutosh Wadhwa[6], Rene Edgar Condori[6], Yu Li[6], Richard Franka[6], Magid Halim[3], Rodney Willoughby[7]

Affiliations: [1] Liver Transplantation, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [2] Medicine, Alfaisal University, Riyadh, Saudi Arabia; [3] King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [4] King Saud University, Riyadh, Saudi Arabia; [5] Hamed Al Essa Organ Transplant Centre, Ibn Sina Hospital, Kuwait; [6] Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, Atlanta, Georgia, USA; Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Abstract: Background: There have been 10 outbreaks of rabies following solid organ or corneal transplantation. In December 2014, 4 organ recipients died in 2 countries. Methods: Chart review and verbal autopsy. Serum and CSF detection of rabies-specific antibodies by IFA and RFFIT. Detection of rabies virus antigen by DFA. Hemi-nested RT-PCR amplified the rabies virus nucleoprotein (N) gene. Phylogenetic analysis compared virus from the recipients with variants circulating in Middle East and Asia. Results: A 28 year old donor presented with chest infection, acute respiratory distress syndrome, cardiac arrest, and seizure. CT of the head was unremarkable. CSF culture was negative. A kidney recipient developed encephalitis within 2 months of transplantation and died. The second kidney recipient developed symptoms a week later and died. The family of the donor confirmed that he was bitten by a domestic dog in India 2 months prior to his death and did not receive prophylaxis; the dog died. The heart recipient had died after cardiac arrest with prodromal neuropsychiatric symptoms. The liver recipient developed drooling and hydrophobia, was treated with the Milwaukee protocol, and died after 34 days. Ante mortem brain biopsy showed Negri bodies. Rabies RNA was detected in brain and saliva. Rabies antibodies were detected in serum and CSF. Rabies was confirmed in the donor by RT-PCR of explanted cornea. The whole N gene sequences of rabies virus from recipient and donor were 99% identical, belonging to the clade of rabies virus variants circulating in India. Deceased kidney recipients had no rabies antibodies. Corneas were explanted from 2 recipients who received prophylaxis; they remain well. Thirty-three family/household contacts and 249 healthcare workers were assessed for exposure risk, with 7 workers and 1 family member receiving PEP. Conclusion: Avoidance of transmission of rare pathogens requires wide bandwidth diagnostics and rapid-cycle surveillance within transplantation networks.

Name: Sergio Recuenco-Cabrera

OP14

Email: sergio.recuenco@gmail.com

Session: Human Rabies and Prophylaxis

Country: Peru

Title: High risk in silence: Massive PEP response for human rabies outbreaks in apparently new areas

Authors: Sergio Recuenco-Cabrera[1], Victor Fiestas Solorzano[1], Oswaldo Cabanillas Angulo[2], Ricardo Lopez Ingunza[1], Albina Diaz[1], Cinthia Rojas Torres[3], Hermann Silva[3]

Affiliations: [1] National Institute of Health, Lima, Peru; [2] National Zoonosis Strategy, MINSA, Lima, Peru; [3] Loreto Regional Health Management, Iquitos, Peru

Abstract: Vampire bat rabies is enzootic in the Peruvian Amazon River Basin. Animal rabies surveillance is biased to areas with significant concentration of cattle so rabies circulation evidence is lacking in remote and poor areas appearing as silent for rabies. In May 2015, a new human rabies outbreak occurred in Soledad, a remote village in the Curaray River, Loreto, Peru. A 7-year boy with encephalitis was referred to the Loreto Regional Hospital in Iquitos. Rabies was confirmed at the Viral Zoonosis Laboratory-NIH Peru, and triggered an outbreak investigation. The patient died later at 26 days after onset. Two additional suspected rabies deaths were identified from the same village. The community owned 20 cattle, from them, 10 died approximately one month earlier with a paralytic neurological syndrome. Two neighboring villages also had dead cattle in recent weeks. Frequent exposures to bat bites, lack of information on rabies, and vulnerable living conditions were common in all villages. An immediate plan for massive rabies post-exposure prophylaxis (PEP) for all population in the three villages was scheduled simultaneously to the outbreak investigation. A response brigade bringing purified Vero cell rabies vaccine (PVRV) was deployed immediately by plane and boat to the three villages. Approximately 500 people initiated PEP and followed to completion. Further massive rabies Pre-Exposure (PreEP) was schedule for remaining villages along the Curaray River for upcoming weeks. No more human cases were identified, no adverse reactions to the vaccine were reported. Permanent policies for use of mass PEP and PreEP in high risk areas need to be developed recognizing the level of risk despite the lack of evidence for rabies circulation

Name: William Thomas

OP15

Email: william.thomas@umassmed.edu

Session: Human Rabies and Prophylaxis

Country: United States

Title: Epitope analysis to predict rabies virus (RABV) sensitivity to human monoclonal antibodies

Authors: Yang Wang[1], Heidi Smith[1], Monir Ejemel[1], Ashutosh Wadhwa[2], Lauren Greenberg[2], William C Carson[2], Todd G Smith[2], Roland Davis[3], Adam Buzby[1], Susan Moore[3], Deborah C Molrine[1], Mark J Klempner[1], William D Thomas Jr[1]

Affiliations: [1] MassBiologics of the University of Massachusetts Medical School, Boston, Massachusetts, USA; [2] Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [3] Kansas State University Rabies Laboratory, Manhattan, Kansas, USA

Abstract: An effective human monoclonal antibody (HuMAb) cocktail to replace HRIG for post-exposure prophylaxis requires HuMAbs with unique epitopes capable of neutralizing relevant RABV isolates. The RABV G glycoprotein (G) epitopes recognized by two HuMabs (RAB1 and RAB2) have been identified by sequencing RABV's resistant in the rapid fluorescent focus inhibition test (RFFIT). RAB1 laboratory escape mutants had changes at N336D and R346K in antigenic site III and the RAB2 escape mutant (E33K) mapped to antigenic site II. No escape mutants could be isolated selecting with a RAB1/RAB2 cocktail (R172). RFFITs were also performed against street isolates to identify other critical residues for RAB1 or RAB2 binding. RAB1 exhibited reduced activity against a Florida raccoon isolate with the change R333Q and was unable to neutralize a bat isolate with the lab-acquired alteration I338T; both isolates were neutralized by RAB2. RAB2's reduced activity against Lasiurus bat isolates with the change E33D confirmed the relevance of that position. Lentiviruses pseudotyped with G (RABVpp) from ERA were made to confirm the importance of these residues to RAB1 or RAB2 binding. Peru bat isolate RABVpp (E33E, N336D, R346K) were resistant to RAB1 but sensitive to RAB2 as predicted. RAB1 activity against RABVpp with changes at positions 333, 336, 338 or 346 was significantly reduced, while RAB2 neutralization of RABVpp with changes at position 33 was also reduced. R172 neutralized all RABV isolates with variations in these residues when tested in either RFFIT or RABVpp. FACS analysis of cells expressing ERA G with alanine mutants in antigenic sites II (32-46,198-200) and III (332-348) was used to rule out other changes that might impact binding. Since variation in positions 333, 336, 338 and 346 affected RAB1 binding and changes in position 33 affected RAB2 binding, sequences of these residues can be used to predict sensitivity of isolates.

Name: Eric Tsao

OP16

Email: etsao@synermore.com

Session: Human Rabies and Prophylaxis

Country: United States

Title: Development of anti-rabies MAbs for post-exposure prophylaxis

Authors: Eric Tsao

Affiliations: Chief Executive Officer, Synermore Biologics Co., Ltd., Taipei, Taiwan

Abstract: SYN023 is a mixture of two anti-rabies humanized monoclonal IgG1κ antibodies which bind to distinct and non-overlapping antigenic sites on the rabies virus glycoprotein. The proposed indication for SYN023 is the post-exposure prophylaxis of rabies virus infection, in conjunction with rabies vaccine. SYN023 has been shown to neutralize more than 15 contemporary clinical isolates of rabies viruses collected in China, and the 10 predominant strains in the US. Protection against virus challenges was demonstrated in various animal models. The development, manufacturing, as well as results from in vitro and in vivo studies will be presented.

Name: Robert Rudd

OP17

Email: robert.rudd@health.ny.gov

Session: Twenty-Five Years of Oral Rabies Vaccination in the U.S. Symposium

Country: United States

Title: Early conceptual development of oral rabies vaccination in New York State

Authors: Robert J. Rudd

Affiliations: New York State Department of Health, Slingerlands, New York, USA

Abstract: Twenty years before the first V-RG oral rabies vaccine were placed on the landscape, research was underway that would lay the groundwork for this program. The work was occurring in Upstate New York, ten miles outside of Albany. Perhaps it was serendipity that led Dr. George Baer to Albany and the New York State Health Department. Dr. Baer started his career in public health with CDC via the EIS, and was assigned to the New York State Health Department in Albany, where he focused upon brucellosis, psittacosis, and rabies. While in Albany Dr. Baer met Dr. John Debbie, a wildlife veterinarian and Director of the New York Rabies Laboratory. At the same time in Albany, the Department of Environmental Conservation wildlife researchers were maintaining a captive colony of red foxes as part of an unrelated study. Perhaps, spurred on by the success of oral vaccines to another single stranded RNA virus, polio, Dr. Baer sought to attempt the same success in wildlife. This presentation will describe these “early days” of oral rabies vaccination.

Name: Florence Cliquet

OP18

Email: florence.cliquet@anses.fr

Session: Twenty-Five Years of Oral Rabies Vaccination in the U.S. Symposium

Country: France

Title: First operational use of V-RG in Europe

Authors: F Cliquet[1], MFA Aubert[2], B Brochier[3]

Affiliations: [1] ANSES, Nancy Laboratory for Rabies and Wildlife, Malzéville, France; [2] Formerly ANSES, Laboratory for Rabies and Wildlife, Malzéville, France, then Laboratory for Bee Health, Sophia Antipolis, France, now independent expert; [3] Scientific Institute for Public Health, National Reference Laboratory for Rabies, Brussels, Belgium.

Abstract: This paper is dedicated to the memory of Dr Louis Andral, Dr Jean Blancou, Dr Eric Masson, Pr Paul Pierre Pastoret and Dr Franck Wolff. In the 1970s, the depopulation of red foxes (*Vulpes vulpes*), main reservoir and vector of rabies in Europe, was the only measure available for limiting the virus transmission in wildlife. Research studies for oral vaccines were developed at the same time, although the use of live attenuated vaccines was banned in Europe. A WHO-leaded international collaboration was developed to exchange on efficacy and safety results obtained with newly developed vaccines. The first field trial of vaccine bait release in Europe was carried out in 1978 in Switzerland with a SAD attenuated strain of rabies virus distributed for foxes in chicken heads. The design of safer vaccines was encouraged and the recombinant vaccinia virus expressing the glycoprotein gene (V-RG) was developed in 1983 within a joint collaboration between the Wistar Institute, Transgène S.A. and the Rhône Mérieux Company. This vaccine was extensively tested in laboratory and field conditions in Europe, particularly in Belgium and in France, and also in USA prior to large-scale use. Efficacy of V-RG was extensively demonstrated in red fox and in raccoon dog including long-term protection and immunisation of cubs despite maternal antibodies. Many experiments demonstrated that V-RG was apathogenic for target and numerous non-target wild and domestic species. Its genetic stability was also assessed in mice and foxes as well as the absence of virus excretion and horizontal transmission. The heat stability was assessed during a multisite European trial. The attractiveness and shape of the bait matrix was investigated in caged and free-ranging foxes and raccoon dogs. In 1986, joint vaccination campaigns using V-RG were carried out in Luxembourg, Belgium and France over small areas, and then progressively extended to a larger scale. The V-RG was the only vaccine used in Luxembourg (1992-1999) and Belgium (1989-1999). France used it from 1989 to 2003 and in 2005. After 2000 and 2001, those bait distributions aimed at preventing contamination from the borders. Several strategies were tested to optimise the efficiency of the campaigns. In France, It was shown that V-RG can eliminate rabies from large areas after two campaigns only. In 1993, all laboratory and field trial results led to a European Commission decision, which has been taking a pivotal role since 1989 in rabies control programmes for “the placing on the market” of the V-RG vaccine. France (2000), Belgium (2001) and Luxembourg (2001) were declared free of terrestrial rabies. A total of 10.5 million V-RG vaccine baits were distributed in these countries from 1989 to 2005.

Name: Charles E Ruppecht

OP19

Email: charles_rupprecht@yahoo.com

Session: Twenty-Five Years of Oral Rabies Vaccination in the U.S. Symposium

Country: United States

Title: Development of a Vaccinia-Rabies Glycoprotein (V-RG) recombinant virus vaccine within North America: an historical retrospective

Authors: CE Rupprecht

Affiliations: The Wistar Institute, Philadelphia, Pennsylvania, USA

Abstract: The development of V-RG in North America was not simply a feat of molecular wizardry unique for the time. Rather, a constellation of concomitant biological, epidemiological, political and technical facets was necessary for the realization of events that transpired from ~ 1977 to 1995, continuing to date. Biosafety formed a back-drop, as vaccine-associated disease from first generation biologics were less than ideal. The choice and manipulation of pox virus vectors was fundamental as a proof of concept. Selection and cloning of the rabies virus gene of interest was pivotal. Determinants for primary target species focused upon foxes, raccoons and skunks for per os delivery and via relevant baits. Major avian and mammalian non-target species were also needed to be tested extensively. A pre-requisite was the finding that, regardless of taxon, no vaccine associated mortality, morbidity, gross or histopathological lesions were detected, regardless of route or concentration. Location of a suitable venue was a major under-taking. Were it not for the mid-Atlantic raccoon rabies epizootic that emerged during the late 1970s, the impetus for the original field trials would have been doubtful, because resistance was high over the concept of the release of a GMO. The later formation of the National Rabies Program within USDA, Wildlife Services, from a dual professional vantage point and the critical acquisition of the federal budgetary support necessary to administer a multi-state and multi-nation project, was instrumental to elevate the basic idea beyond the small scale and the short term. Eventual commercial production and licensure of V-RG (AKA Raboral) assured the release of a pure, potent, safe, stable and efficacious vaccine intended for the oral vaccination of multiple free-ranging carnivores within government-sponsored programs. The global team work entailed a dedicated academic, commercial, NGO and governmental partnership that grew from a singular basic laboratory concept to successful application, with more than 160 million doses of V-RG distributed over a 25 year span.

Name: Joanne Maki

OP20

Email: joanne.maki@merial.com

Session: Twenty-Five Years of Oral Rabies Vaccination in the U.S. Symposium

Country: United States

Title: Wildlife ORV products and programs in the United States: A manufacturer's perspective

Authors: Joanne Maki[1] and Emily Lankau[2]

Affiliations: [1]Veterinary Public Health, Merial, Inc., Athens, Georgia, USA; [2] LandCow Consulting, Madison, Wisconsin, USA (landcow.ecohealth@gmail.com)

Abstract: RABORAL V-RG® was first used in Europe during the 1980s to control and eliminate rabies in red fox populations in France, Belgium and Luxembourg. This year marks the 25th anniversary of its first use in the United States for wildlife rabies control and prevention. Experimental US field trials targeting raccoons during 1990-1991 led to the first US oral rabies vaccination (ORV) program in Cape May, New Jersey during 1992. Soon other wildlife rabies control programs followed at the federal, state, and local levels. Major milestones of US ORV history included: the establishment of the USDA-Wildlife Services program to prevent the western spread of raccoon rabies beyond the Appalachian Mountains; smaller programs, such as the Long Island, NY program, celebrating local successes in reducing or eliminating the prevalence of raccoon rabies in the eastern US; and RABORAL V-RG use for elimination of the canine strain of rabies from Texas coyote populations and near-elimination of the Texas gray fox virus variant from the United States. Environmental release of this recombinant vaccine was the first of many challenges overcome by public-private partnerships which led to the efficient production and national distribution of this unique vaccine in sufficient volumes to address rabies in multiple wildlife species. The production process and delivery pipeline for RABORAL V-RG have grown and evolved in partnership with the needs of US ORV program customers to ensure supply for contingency actions and efficient cold-chain delivery to often remote locations. Merial remains supportive of the evolving US wildlife ORV program as field parameters and product needs shift from creating barriers to elimination of raccoon and skunk rabies variants from the US. Merial's commitment to rabies control extends well beyond simply providing vaccine, technical and logistical support. Sanofi-Pasteur and Merial are global One Health leaders in the fight against rabies.

Name: Richard Chipman

OP21

Email: richard.b.chipman@aphis.usda.gov

Session: Twenty-Five Years of Oral Rabies Vaccination in the U.S. Symposium

Country: United States

Title: Current ORV landscape in the U.S. and thoughts on the future

Authors: Richard B Chipman, Kathleen M Nelson, Timothy P Algeo, Dennis Slate

Affiliations: US Department of Agriculture, Wildlife Services, National Rabies Management Program, Concord, New Hampshire, USA

Abstract: Oral rabies vaccination (ORV) has become an accepted adjunct to conventional rabies prevention and control in the U.S., enabling a collaborative management focus at the landscape level in specific wildlife rabies reservoir species. ORV successes in the coyote, gray fox and raccoon have depended on multidisciplinary collaboration in strategic planning and implementation across integrated program functions of enhanced rabies surveillance, management (ORV and contingency actions), monitoring, and research. Currently, the ORV is a mosaic of long-term management to: prevent reemergence of canine rabies from Mexico, eliminate a variant of gray fox rabies unique to Texas, and prevent raccoon rabies from spreading appreciably beyond its current distribution. Field experiments are ongoing that explore vaccine-bait and strategy combinations that could lead to elimination. RABORAL V-RG® has been critical to the historic and present fight against wildlife rabies; however, V-RG baits combined with distribution strategies have not produced sero-prevalence levels required to break the rabies transmission cycles in raccoons or skunks. ONRAB field trials show promise based on high raccoon sero-prevalence in rural areas, but urban-suburban environments present challenges. Studies documenting sero-conversion associated with 150 VRG baits/km^2 in raccoons and 300 ONRAB baits/km^2 in skunks are underway, as is research toward an ORV field trial in mongoose in Puerto Rico. Given the breadth of research questions, future studies will focus on wildlife and rabies ecology, vaccine baits and ORV strategies critical to program successes and sustainability. Economic analyses are essential to provide rabies management costs in the context of financial and quality of life benefits. Lastly, regulatory compliance and strategic planning to protect human and animal safety, and the environment, and maintaining a viable, diverse international coalition of government, university, organization and private sector collaborators and stakeholders provides the backbone for effective ORV and achieving rabies management objectives in the U.S.

Name: Richard Bowen

OP22

Email: rbowen@rams.colostate.edu

Session: Keynote Speaker

Country: United States

Title: Significance of anti-rabies virus antibodies in healthy bats: evidence of repeated, sub-infectious exposures to rabies virus?

Authors: RA Bowen[1], TJ O'Shea[2], CE Rupprecht[3]

Affiliations: [1] Colorado State University, Department of Biomedical Sciences, Animal Reproduction and Biotechnology Laboratory, Fort Collins, Colorado, USA; [2] United States Geological Survey, Fort Collins, Colorado, USA; [3] The Wistar Institute, Philadelphia, Pennsylvania, USA

Abstract: The presence of antiviral antibodies in healthy hosts is typically interpreted as evidence of previous infection followed either by clearance of the pathogen or in association with establishment of a persistent or latent infection. Infection with rabies virus is generally associated with lethal disease, yet several investigators have reported healthy bats having anti-rabies virus neutralizing antibodies. As part of studies to evaluate mechanisms of rabies virus maintenance in an urban ecosystem, we measured anti-rabies virus antibodies in serum of 2332 big brown bats (Eptesicus fuscus) captured and re-captured around Fort Collins, Colorado. Seroprevalence increased with age among females sampled yearly for 5 years and was variable among roosts. Multiple bats remained healthy one to four years after the original testing and viral RNA was not detected in oropharygeal swabs from 261 seropositive bats sampled. Rabies virus infection is endemic in the population of bats studied and we hypothesize that bats become immunized but not productively infected as a result of repeated, sub-infectious exposure to rabies virus from other bats in their colony that develop clinical disease.

Name: Ai-Ping Hsu

OP24

Email: aphsu@mail.nvri.gov.tw

Session: Pathogenesis and Molecular Epidemiology

Country: Taiwan

Title: Replication properties of Taiwan ferret badger rabies virus in mice

Authors: Ai-Ping Hsu[1,2], Chun-Hsien Tseng[1], Yu-Hua Shih[1,2], Chung-Hsi Chou[2], Florence Cliquet[3], Hsiang-Jung Tsai[1,2]

Affiliations: [1] Animal Health Research Institute, Council of Agriculture, New Taipei City, Taiwan; [2] National Taiwan University, School of Veterinary Medicine, Taipei, Taiwan; [3] ANSES, Nancy Laboratory for Rabies and Wildlife, Malzéville, France

Abstract: Taiwan has been considered as rabies-free since 1959. However, investigating death reasons of wild carnivores in 2013 unexpectedly revealed outbreaks of Taiwan ferret badger rabies, and which further changed the rabies status of Taiwan suddenly. A total number of 475 positive cases have been recorded since the first case in 2013 to the end of June 2015, most of them in the ferret badgers in eastern, middle, and southern parts of Taiwan, especially in Taitung, Nantou, and Taichung counties. Genome sequencing and phylogenetic analysis of 10 strains of Taiwan ferret badger rabies virus (RABV-TWFB) isolated in 2013 in different areas of above parts of Taiwan revealed 3 genetic groups exist in RABV-TWFB populations, and those are highly related and even restricted to the geographical distributions. In this study, MIC LD50 titers of RABV-TWFB of the 3 groups were first evaluated by using the original animal brain homogenates, and mortality developing patterns were observed. From the results displayed, all the RABV-TWFB of the 3 genetic groups showed lower MIC LD50 titers as 10^1.5 MIC LD50/g-organ around in compared to other street rabies virus strains in recorded reviews. On the other hand, late, slow, and long disease and mortality developing patterns were found as well after intracranial inoculations of RABV-TWFB into mice. All the above featured RABV-TWFB exhibited a much different pathogenicity in mice in comparison with other rabies virus strains. From the views of this study, RABV-TWFB will be further evaluated in ferret badgers for characterizing pathogenicity properties in the futures.

Name: Susan Nadin-Davis

OP25

Email: Susan.Nadin-Davis@inspection.gc.ca

Session: Pathogenesis and Molecular Epidemiology

Country: Canada

Title: Genomic molecular epidemiology of raccoon rabies using next generation sequencing

Authors: Susan A Nadin-Davis[1], Roman Biek[2], Les Real[3]

Affiliations: [1] Canadian Food Inspection Agency, Animal Health Microbiology Research, Ottawa, Ontario, Canada; [2] University of Glasgow, College of Medical, Veterinary and Life Sciences, Glasgow, United Kingdom; [3] Emory University, Department of Biology, Atlanta, Georgia, USA

Abstract: The raccoon rabies virus (RRV) strain continues to pose significant health concerns and a large economic burden for much of eastern North America. Models of disease spread can help to inform more efficient cost-effective control efforts. One current modelling initiative aims to use the genetic signatures identified throughout the viral genome as part of a model building process to better understand the factors that impact RRV spread. To facilitate this study, a protocol that facilitates the generation of whole genome sequences of hundreds of viral samples has been developed. The traditional methods of RNA extraction and RT-PCR were modified so as to amplify the complete rabies virus genome as a small number of overlapping amplicons. Sequencing of products from up to 96 viral isolates at a time is performed in parallel using an Illumina MiSeq instrument. To date over 600 whole viral sequences have been generated during the development of this procedure. The value of a whole genome sequencing approach to study the molecular epidemiology of RRV is illustrated using samples recovered from the state of Vermont and neighboring regions of adjoining US states. Whole genome sequence analysis led to the identification of eight viral lineages which were locally distributed around Vermont State. Spread of this rabies virus strain across state lines was identified multiple times especially across the New York - Vermont state boundary and the impact of landscape features on variant spread was evident. This study clearly demonstrated the far greater robustness of phylogenetic linkages predicted from whole genome sequences compared to those suggested from individual genes when analysing viral populations with a low overall genetic diversity. With little or no modification to the PCR primers this same workflow can be applied to detailed study of other rabies virus variants world-wide.

Name: Rene Edgar Condori Condori

OP26

Email: hws5@cdc.gov

Session: Pathogenesis and Molecular Epidemiology

Country: United States

Title: Recognition of a novel rabies virus variant in grey fox

Authors: Rene Edgar Condori Condori[1], Paul Ettestad[2], Ashutosh Wadhwa1, Ginny Emerson[1], Mathew Mauldin[1], Jeff Doty1, Adam Aragon[2], Darin Carroll[1], Ryan Wallace[1], Yu Li[1], Andres Velasco-Villa[1]

Affiliations: [1] Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, Atlanta, Georgia, USA; [2] New Mexico Department of Health, Santa Fe, New Mexico, USA

Abstract: Since dog related rabies has been eliminated in the USA, terrestrial carnivores and bats play an important role in rabies transmission and maintenance. Currently, different variants of the Rabies Virus (RABV) were identified; each being mammal specific. On April 20, 2015 an incident occurred in Lincoln County, New Mexico, in which a woman was bitten by a wild gray fox. The animal was captured and euthanized, testing positive for RABV. The patient received timely post exposure prophylaxis. With the aim to determine the host of the RABV involved in this case, the nucleoprotein gene was targeted. Sequence analysis revealed a novel RABV variant not previously detected in the USA and throughout the Americas. Retrospective positive samples of bats, foxes and skunks from New Mexico were sequenced to elucidate the reservoir of such novel RABV. In addition, sequences of RABV associated to established bat and terrestrial rabies reservoir hosts throughout the Americas were used to reconstruct a robust phylogenetic tree. Final analysis supported that the RABV found in the gray fox is a new variant which is closely related to those circulating in tree bats. Recent and historic evidence suggest that RABV is capable of jumping from one host to another, successfully establishing emerging rabies enzootics in novel reservoir host. Occurrence of these events in wildlife is becoming more frequent posing new challenges and opportunities for rabies control and its prevention in humans. In conclusion, this study corroborated circulation of long term rabies enzootics in New Mexico in which main RABV variants involved are Arizona grey fox, South Central Skunk, Eptesicus fuscus and Myotis. Enhanced active surveillance in bats and terrestrial carnivores populations in Lincoln county and neighboring areas should provide clues about the reservoir host of this new rabies variant and for how long has been circulating in the region.

Name: Patricia Arias-Orozco

OP27

Email: aoe.patricia@gmail.com

Session: George M. Baer Latin American Investigators Award Winner

Country: El Salvador

Title: Canine Rabies in El Salvador 2005-2014: Spatial analysis of control and the influence of social determinants

Authors: P Arias-Orozco[1], F Bastida-Gonzáles[1,2], L Cruz[3], J Villatoro, E Espinoza[3], P Zárate Segura[1], S Recuenco[4]

Affiliations: [1] ESM-UPIBI, Instituto Politécnico Nacional, México; [2] Instituto de Salud del Estado de México, Toluca, Estado de México; [3] Ministerio de Salud de El Salvador, El Salvador; [4] Centro Nacional de Salud Pública, INS, Lima, Perú

Abstract: Canine rabies incidence has been achieved in El Salvador with the implementation of control strategies but it is unknown which factors could be associated to its spatial patterns. These interventions have decreased in some regions and report an increase in levels of violence and poverty. The "maras" controlled these areas of violence which makes interventions difficult to implement. The main objective was to identify spatiotemporal patterns and their association with social determinants, poverty and insecurity, in El Salvador 2005-2014. A Poisson-regression model was used and a spatial scan statistic to identify statistically significant clusters using three modeling approaches: Space-time permutation, Bernoulli and Poisson unadjusted/adjusted with covariates. A search for cold spots was performed to characterize areas of lowest rates for canine rabies. The results showed high risk of rabies was associated with poverty (RR=7.74). Insecurity was significant but with negative association (RR=0.663), possibly due to uncontrolled bias. Higher risk townships were concentrated in the central and east regions. Significant clusters were also detected in these regions. Adjusted analysis allows the discussion of possible causes of high risk zone. Cold spots analysis identified a low rate area in the Central and Eastern Region when >70% of dog vaccination coverage was achieved. Poverty and insecurity were significant and represent a novel approach to evaluate rabies control interventions in these regions. The high risk areas identified were correlated with the hot spots found with scan statistics, with further cluster analysis adjusted to covariates the influence of social factors was confirmed. These findings demonstrate the decrease of canine rabies incidence when interventions were progressively implemented. The analysis of social factors provide national authorities with valuable information for decision-making when planning and implementing new canine rabies control intervention strategies directed towards areas of social conflict, and encourage to maintain surveillance and continue control strategies after canine rabies control to prevent rabies reintroduction, based in epidemiological evidence profiling risk areas for reintroduction.

Name: Charles Rupprecht

OP28

Email: charles_rupprecht@yahoo.com

Session: Ted Talks

Country: United States

Title: Are we 'batty' to consider rabies prevention and control among the Chiroptera?

Authors: CE Rupprecht

Affiliations: The Wistar Institute, Philadelphia, Pennsylvania, USA

Abstract: Within an evolutionary and epizootiological context, bats are major reservoirs, vectors and victims of rabies, responsible for perpetuation of this zoonosis, independently of other mammals. No serious discussions have occurred as to potential prevention and control of lyssaviruses among bat populations. One obvious solution is to continue to employ ignorance, as the status quo, focusing only upon the low hanging public health and veterinary fruit of people, pets and a few assorted wild carnivores. At least continued concentration to the known and the proven is much more benign than the disastrous idea of widespread lethal population reduction, not supported from ecological, economic or ethical grounds. Yet, if solutions are being sought for White Nose Syndrome, which has killed millions of bats, from the realm of conservation biology, perhaps collective scientific aim should be a bit higher. For certain genera, bats remain captive subjects in limited spatial sanctuaries over long seasonal periods. Methods could be honed for focal acoustic beacons to attract rabid individuals for capture, to begin dilution of landscapes from super spreaders. Biotechnology exists for the development and delivery of unconventional but pure, potent, safe and efficacious biologics to the unaffected masses prior to viral exposure. Clearly, besides lyssaviruses, in association with a plethora of other pathogens, including coronaviruses, filoviruses and henipaviruses, a true need for more immediate practical solutions might evolve sooner than fathomed. If so, yesterday's science fiction becomes tomorrow's headline of yet another deadly emerging infectious disease with mankind unprepared. Better to muse and model today over the possible in advance of last minute reckoning. Eventually, in a truly holistic, trans-disciplinary approach to disease management, within an ultimate One Health philosophy, researchers will have to turn necessarily, not only to humans, domestic animals and certain species of other terrestrial wildlife, but eventually extend consideration to bat populations themselves, or at least begin with certain taxa as targets, if the concept of true rabies eradication will ever begin to approach fruition.

OP29

Name: Thomas Müller

Email: thomas.mueller@fli.bund.de

Session: Ted Talks

Country: Germany

Title: Monitoring of ORV campaigns – essential or a waste of time and money?

Authors: Thomas Müller

Affiliations: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald - Insel Riems, Germany

Abstract: Opening statement: Looking back at years of monitoring of oral vaccination campaigns in Europe and North America I am wondering if we are drowning in testing for the sake of testing. I think we have become quite content and institutionalized in our approach in producing figures for annual summary reports of oral rabies management programs just because it is required of us, but then subsequently need a crystal ball to interpret the data. Oral rabies vaccination (ORV) of wildlife has been a milestone for the control and elimination of wildlife rabies in large parts of Europe and North America. Next to rabies surveillance, monitoring of vaccination campaigns is considered a key element for the evaluation of wildlife ORV programs. The latter comprises the assessment of both (i) bait-uptake by detection of a biomarker (tetracycline - TC) in bone and tooth tissues and (ii) seroconversion rate by providing evidence for rabies specific antibodies (Ab) in the target species with (iii) aging animals to estimate vaccination coverage in juvenile and adult subpopulations. In contrast to rabies surveillance, sampling for ORV monitoring focusses on healthy target animals that are specifically sampled for the purpose. As a result, every year tens of thousands of animals from vaccination areas are tested resulting in considerable annual costs. Often this is done in a sort of routine checkbox approach to meet international recommendations. Depending on the results, ORV campaigns are construed as being highly or less effective. However, reality is anything but real. More than 120 000 kilograms of TC have been distributed in the environment during ORV campaigns, however, estimation of the true post-vaccination bait-uptake in the target population is limited due to life-long marker and high TC background levels. In contrast to vaccines efficacy studies for licensing of where seroconversion is subordinate, strangely it is considered top priority in ORV monitoring. Here, determination of seroconversion mainly relies on serum neutralization with empirically chosen or supposed cut-offs for individual target species. Oddly, we use VNAs to draw conclusions on the level of protection in the target species, while the full immune response is largely ignored. Matters go even more complicated considering the lack of diagnostic standardization, quality of specimen, inadequate sample sizes, unavoidable spatial and temporal sampling biases, lack of context and often inappropriate analysis. As a consequence, there is high variability in bait uptake and seroconversion rates on a regional level and often results do not fit what rabies surveillance data suggest. As monitoring results can hardly be compared and interpreted, the question is what does this actually tell us? There is reason to believe that monitoring data only had a very limited impact on the implemented ORV strategy over the past 35 years. So, is there any informative value ORV monitoring can provide in addition to rabies surveillance? Hence, is the current approach meaningful, timely and worth the effort? Taking new quality features for ORV campaigns into account, actually, it is time to reconsider our approach by trying to find answers to what is really essential in ORV monitoring and reinvest the saved money in a better way.

Name: Christine Fehlner-Gardiner

OP30

Email: Christine.Fehlner-Gardiner@inspection.gc.ca

Session: Ted Talks

Country: Canada

Title: That was then, this is now – paradigm shifts since I started working in rabies

Authors: Christine Fehlner-Gardiner

Affiliations: Canadian Food Inspection Agency, Ottawa Laboratory-Fallowfield, Ottawa, Ontario, Canada

Abstract: I began my career in rabies when I joined Alex Wandeler's lab at the Canadian Food Inspection Agency (CFIA) in 2002, providing research support to various collaborative projects. Promoted to Research Scientist in 2004, and now head of the rabies lab at CFIA, my time in the rabies field has been an ongoing education, involving amazing mentorship from a great many people, a lot of self-study, and the occasional baptism by fire. Through this work I have had the opportunity to be involved in diverse aspects of rabies: oral vaccination of wildlife; diagnostics for animal and human cases; serology; molecular biology; national and international relations; and public outreach, among others. During this time, developments in the field have challenged some of the concepts to which I was introduced when I began my career. We have seen experimental treatments resulting in survival from human rabies; field application and licensing of new oral vaccines for control of rabies in wildlife; progress in the application of new diagnostic tests, both simple and advanced platforms; and a renewed focus on inter-sectoral co-operation in the fight towards the elimination of human rabies. In this talk I would like to highlight what I consider to be some of the interesting changes in thinking over the last decade that are shaping the approaches to the prevention and management rabies in both animals and people, and what impact these may have as we collectively work toward "Making It Work Better" in the 21st century.

Name: Ad Vos

OP31

Email: Ad.Vos@idt-biologika.de

Session: Ted Talks

Country: Germany

Title: Oral vaccination of dogs against rabies; Ubi eras?

Authors: Ad Vos

Affiliations: IDT Biologika, Dessau-Rosslau,, Germany

Abstract: The core of the global rabies problem is the free-roaming dog population, owned or ownerless. The majority (95%) of the human rabies cases are a direct result of a dog bite and account for an estimated 59,000 deaths a year, mainly in developing countries. Besides human casualties, dogs are responsible for most rabies cases in livestock. Sustained spill-over infection from dogs to wildlife species has been known for decades; e.g. mongoose rabies in the Caribbean. Furthermore, rabies among free-roaming dogs forms a serious threat to certain highly endangered species like the Ethiopian wolves. Hence, from a global One Health perspective, dog rabies control should have the highest priority. Vaccination of these animals is the most cost-effective means of preventing and eradicating dog-mediated rabies. Unfortunately, mass dog vaccination campaigns have often failed or were not sustainable. In certain areas, it is just not possible to reach a sufficient number of dogs by parenteral vaccination. In the late 1980s, this problem was already identified and based on the great successes obtained by oral vaccination of foxes against rabies in Europe and North America the possibility of oral vaccination of dogs (OVD) against rabies was investigated. OVD field studies were conducted showing its feasibility. However, at the beginning of the 21st Century the concept of OVD completely disappeared from the agenda. Was it the lack of safe and efficacious vaccines or suitable baits on the one hand, or was it perceived Cost of Goods, lack of economical sustainable funding or political will? New highly efficient and safe vaccine candidates and bait constructs have become available at affordable costs. Therefore, it is suggested to bring OVD back on the agenda and incorporate the OVD concept together with parenteral vaccination into a sustainable dog-mediated rabies eradication strategy.

Name: Larry Clark

OP32

Email: larry.clark@aphis.usda.gov

Session: Ted Talks

Country: United States

Title: Wildlife Services and 10 billion other people

Authors: Larry Clark

Affiliations: US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA

Abstract: By the year 2050 the earth will have 10 billion people. The strain on the world's resources continues to grow as we hurl towards this benchmark. For example, projections of world hunger severity indices and production suggest that most countries will be in an agricultural and human food energy deficit, and that there will be only four countries in an agricultural surplus status and exporting products. Concepts of One Health and Global Food Security attempt to frame a healthy world ecosystem in terms of an intersection of activities among human health, animal health and environmental health; each with equal weightings. But the reality is that environmental health will suffer greatly as the world struggles to meet the energy and nutritional demands of an ever needed human population. Agricultural product will intensify putting a strain on shrinking environmental resources, in essence creating human-wildlife conflicts in ways we have yet to imagine. The Wildlife Services mission must remain flexible and embrace these new challenges, developing new management schemes, new methods, and new ways to estimate the value of natural systems and portray those activities in currencies and valuations that will be germane to policy makers and the private sector. These are large and daunting tasks, but agencies and organizations should not become paralyzed into inaction because of the scale and scope of the problems we face. By doing our part, by solving problems within our expertise, by facing up to the challenges with a positive "can-do" attitude small changes can, in aggregate, have profound collective impact. Wildlife Services is up to those challenges both in terms of research and operational implementation. I will present a case study on how Wildlife Services parsed out the current avian influenza situation into a manageable action plan for wildlife risk and management, but this is only one of day to day approaches we take toward achieving One Health and Global Food Security.

Name: Craig Hooper

OP33

Email: Douglas.Hooper@jefferson.edu

Session: Ted Talks

Country: United States

Title: Are you protected from rabies? Why human rabies vaccines are not entirely protective and rabies post-exposure prophylaxis (PEP) can fail

Authors: D. Craig Hooper

Affiliations: Thomas Jefferson University, Department of Cancer Biology, Philadelphia, Pennsylvania, USA

Abstract: Human rabies vaccines are highly effective at eliciting substantial titers of virus neutralizing antibodies (VNA) in most people. Consequently those who have been immunized against rabies due to occupational risks or have promptly received PEP due to the possibility of exposure believe they are fully protected against rabies infection. The nature of the immune response induced by current human inactivated rabies vaccines suggests otherwise. Inactivated vaccines are processed by exogenous antigen presenting pathways that induce type 2 immune responses, characterized by Th2 cell activity and high level antibody production. Type 1 immunity, mediated by Th1 cells, is more effective in clearing virus from infected cells. Moreover for an as yet unknown reason, Th2 cells are inactive in rabies-infected neural tissues. Thus an infecting virus is only susceptible to type 2 mechanisms near its initial point of entry and only if there is sufficient local VNA to prevent the virus from reaching nervous tissue. However, once the virus reaches spinal cord tissues, VNA and Th2 immune mechanisms are no longer effective and the virus will spread to the CNS with serious consequences. A type 1 immune response generates a combination of factors, antibodies and cytotoxic T cells that is highly effective in antiviral immunity. However, while Th1 cells can function in CNS tissues, wild-type rabies viruses spread through neural tissues without revealing their presence to the immune system. For full protection, a Th1 response together with a mechanism that drives the associated immune effectors into neural tissues are both required. This is true for effective PEP as well as the establishment of immunological memory that can protect against a challenge that reaches neural tissues. Appropriately engineered live-attenuated rabies viruses with the capacity to spread to the CNS can fulfill both criteria by generating a therapeutic Th1 neuroimmune response and immune memory in CNS tissues.

Name: Elodie Kip

OP34

Email: elodie.kip@ugent.be

Session: Immunology and Vaccines

Country: Belgium

Title: Impact of MALT1, an upstream mediator of NF-κB and immune cell activation, on rabies virus disease

Authors: Elodie Kip[1,2,3], Vanessa Suin[1], Jens Staal[2,3], Michael Kalai[1], Rudi Beyaert[2,3], Steven Van Gucht[1,4]

Affiliations: [1] National Reference Centre of Rabies, Viral Diseases, Communicable and Infectious Diseases, Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium; [2] Unit of Molecular Signal Transduction in Inflammation, Inflammation Research Center, VIB, Ghent, Belgium; [3] Ghent University, Department of Biomedical Molecular Biology, Ghent, Belgium; [4] Ghent University, Faculty of Veterinary Medicine, Department of Virology, Parasitology and Immunology, Laboratory of Virology, Ghent, Belgium

Abstract: Rabies virus is a highly neurovirulent RNA virus, which causes about 59000 deaths in humans each year. The paracaspase MALT1 is crucial for immune and inflammatory cell activation by different receptors, including antigen receptors, lectin receptors, and some GPCRs. MALT1 acts both as a scaffold signalling protein and a cysteine protease that cleaves several substrates, promoting NF-KB signaling and specific mRNA stabilization. As a result, MALT1 activation drives the expression of multiple immunoregulatory genes. Mepazine, an inhibitor of MALT1 proteolytic activity, proved recently to have therapeutic effects in B lymphoma and experimental autoimmune encephalomyelitis in mice. Here, we examined the role of MALT1 in the development of rabies disease using MALT1 knock-out (KO) mice or mepazine treatment. Full KO and wild type (WT) control mice were infected intranasally with the virulent rabies strain CVS-11. MALT1 KO significantly slowed down the development of rabies disease (median survival time of 8 versus 12 days). Moreover, viral RNA loads, IL-1β and IFN-γ levels were decreased in the brains of KO mice compared to WT mice. Daily mepazine treatment also delayed the onset of disease, suggesting an involvement of the MALT1 proteolytic activity. We are currently further examining the impact of MALT1 deficiency in mice with conditional KO in specific cell types to better understand the role of MALT1 in rabies pathogenesis. MALT1 and mepazine have a significant impact on the progression of rabies virus infection and disease, which emphasizes the importance of inflammatory/immunological mechanisms on rabies disease development.

Name: Aurore Lebrun

OP35

Email: aurore.lebrun@jefferson.edu

Session: Immunology and Vaccines

Country: United States

Title: A Th1-type immune response and limited changes in blood-brain barrier integrity are required for efficient clearance of RABV from CNS tissue

Authors: Aurore Lebrun, Carla Portocarrero, Rhonda B Kean, D Craig Hooper

Affiliations: Thomas Jefferson University, Department of Cancer Biology, Philadelphia, Pennsylvania, USA

Abstract: Most neurotropic viruses induce inflammation where extensive blood-brain barrier (BBB) permeability and tissue damage leads to central nervous system (CNS) pathology. The BBB protects vulnerable brain tissues from circulating cells and factors but in doing so, limits the delivery of therapeutics into the brain. Little is known about immune cell infiltration into the CNS in the absence of pathological inflammation. Attenuated rabies viruses (RABV) are unique tools to study this process as their clearance involves lymphocyte but no monocyte infiltration into CNS tissues and limited neurovascular permeability. While inflammatory mechanisms that cause BBB permeability are generally associated with the loss of the endothelial tight junction proteins claudin-5, occludin, and ZO-1, we have recently discovered that only ZO-1 coverage is altered during the clearance of attenuated RABV from CNS tissues. Clearance of attenuated RABV from normal mice involves Th1 CD4 T cell activity in the CNS. T-bet-/- mice, which lack Th1 cells, fail to develop fully protective immunity after RABV immunization and consequently do not survive challenge with a lethal virus. Therefore, we concluded that the selective loss of ZO-1 mediates changes in the BBB, sufficient to allow lymphocyte entry into CNS tissues, which in combination with Th1 activity plays a critical role in the clearance of RABV from CNS tissues. Understanding how the BBB may be therapeutically modulated has great significance not only for virus clearance but also for any disease where immune intervention in CNS tissues is desired.

Name: Zhen Fu

OP36

Email: zhenfu@uga.edu

Session: Immunology and Vaccines

Country: United States

Title: The importance of virus neutralizing antibodies (VNA) in clearing rabies virus from the CNS

Authors: Zhen F Fu

Affiliations: University of Georgia, College of Veterinary Medicine, Department of Pathology, Athens, Georgia, USA

Abstract: One of the major hallmarks for rabies is the almost 100% mortality. There is still no proven therapy for clinical rabies despite of the development of Milwaukee protocol. It has been known for many years that most of the rabies patients (>70%) do not develop virus neutralizing antibodies (VNA) at the time of death, which has also been observed in laboratory animals including mice, dogs and skunks after experimental infection with wt rabies virus (RABV). It has been demonstrated that wt RABV is incapable of inducing innate (inflammation, DC activation) and adaptive immunity (VNA), most likely due to the restricted expression of the glycoprotein (G). Thus evasion of the host immunity is one of the important pathogenic mechanisms for rabies. It has been further demonstrated that VNA alone in the periphery is unable to clearing an established RABV infection in the CNS unless present in the CNS. It has been demonstrated that wt RABV in the CNS can be cleared and infected animals saved by direct intracerebral administration of attenuated or recombinant RABV, which not only lead to the production of VNA in the periphery, but also enhance the Blood-brain Barrier (BBB) permeability. It has been further shown in mice that intravenous administration of VNA in combination with chemokines that enhances the BBB permeability can clear wt RABV from the CNS and prevent the development of rabies. With all these new discoveries, is it possible to try such modalities alone or in combination in large animals or even in humans?

Name: Amy Gilbert

OP37

Email: amy.t.gilbert@aphis.usda.gov

Session: Immunology and Vaccines

Country: United States

Title: Immunogenicity of single shot vaccination with TriGAS and GnRH in raccoons and coyotes

Authors: Amy Gilbert[1], Doug Eckery[1], Shylo Johnson[1], Darcy Mora[1], Kurt Vercauteren[1], Milosz Faber[2]

Affiliations: [1] US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA; [2] Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Abstract: Vaccination schemes involving single dose delivery are important in the prevention and control of animal rabies and population management by sterilization. Oral rabies vaccination programs for wildlife originally utilized live attenuated rabies viruses and the first strain used was Street Alabama Dufferin (SAD) Berne. A recombinant virus, SPBN was derived from SAD virus and the pathogenicity was removed by replacing the SPBN G protein with a G protein that encoded an amino acid change at position 333. More recently, a variant of SPBN was created that expresses three copies of the G protein (i.e., TriGAS). The TriGAS product was paired with carrier coupled GnRH (ccGnRH) as a potential single shot immunocontraceptive rabies vaccine. Initial trials with mice revealed that higher anti-GnRH antibodies were stimulated in mice co-inoculated with TriGAS and ccGnRH compared to mice receiving only ccGNRH. In the current study, we tested a single shot co-injection of TriGAS and ccGnRH in raccoons (*Procyon lotor*) and coyotes (*Canis latrans*) to compare with treatments of either TriGAS or ccGnRH alone, or sterile phosphate buffered saline. Animals were bled and swabbed at intervals for at least six months. Antibody titers against GnRH were determined by ELISA, and rabies virus neutralizing antibody (rVNA) titers were determined by modified FAVN. In raccoons and coyotes, rVNA seroconversion was observed in greater than 80% of TriGAS vaccinated animals. No antibody response to GnRH was observed in animals treated with ccGnRH. Higher doses and/or alternate formulations of ccGnRH will need to be considered for a single shot immunocontraceptive rabies vaccine targeting mesocarnivores.

Name: Charles E Rupprecht

OP38

Email: charles_rupprecht@yahoo.com

Session: Immunology and Vaccines

Country: United States

Title: Effector functions of monoclonal antibodies during rabies virus infection

Authors: CE Rupprecht[1] and WE Marissen[2]

Affiliations: [1] LYSSA LLC, Cummings, Georgia, USA; [2] Janssen Vaccines, Leiden, Netherlands

Abstract: Since the late 1970s, multiple neutralizing monoclonal antibodies (Mabs) have been shown to be both broadly cross reactive against diverse rabies virus variants in vitro and safe and effective in rabies postexposure prophylaxis in vivo. Less information is available concerning the potential role of such Mabs in antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). The objective of this research was to compare the mode of action of parent and modified Mabs directed against the rabies virus glycoprotein (G) in an animal model. For two different human Mabs, CR57 and CR4098, leucine-to-alanine (Lala) point mutations were introduced into the heavy chain sequence of the hinge region in the constant (Fc) domain. The Mabs were expressed in PER.C6 cells and purified by protein A affinity chromatography. Antibody integrity was verified by SDS-PAGE, size exclusion chromatography and IEF. Protein concentration was determined by absorbance at 280 nm. Groups of female Syrian hamsters were inoculated on day 0 with rabies virus isolated from an insectivorous bat (*Tadarida brasiliensis*). Two hours later, animals received PBS only (controls), commercial human rabies vaccine only, Mab CR57 plus vaccine, Mab CR57Lala plus vaccine, Mab CR4098 plus vaccine or Mab 4098Lala plus vaccine. Hamsters were observed daily and euthanized when clinical signs of rabies appeared. At necropsy, brains were removed and examined for rabies virus antigens by the DFA test. All controls, as well as animals administered rabies vaccine only, succumbed. Survivorship in the parental groups, administered Mabs CR 4098 or CR 57 (at 12 ug/kg), ranged from 33-58%, whereas mortality in both of the Mab Lala-mutation groups exceeded 83%. Survivorship improved slightly when Mab concentrations were increased to 24 ug/kg. These preliminary experimental data provide insights that, besides antigen binding sites, the role of the Fc region and ADCC/CDC effector functions may also be critical in the protective capacity of anti-G Mabs against a severe rabies virus infection.

Name: Elizabeth McQuade

OP39

Email: emcquade@vet.ksu.edu

Session: Rabies in Domestic Animals

Country: United States

Title: Forgeries of pet export rabies serology reports

Authors: Elizabeth McQuade, Dale Claassen, Susan Moore

Affiliations: Kansas State University Rabies Laboratory, Manhattan, Kansas, USA

Abstract: As the world accelerates in mobility, travel to rabies-free countries continually increases as popular destinations for those accompanied by animal companions. Policies vary among quarantine stations in rabies-free countries. A majority of these destinations require a minimum rabies antibody titer of greater than or equal to 0.50 IU/mL as part of the assurance of adequate vaccination. The Kansas State University Rabies Laboratory, an OIE (World Organisation for Animal Health) approved laboratory, has provided serological rabies testing for pet travel purposes since 2002 by two recognized testing methods: Fluorescent Antibody Virus Neutralization (FAVN) and Rapid Fluorescent Focus Inhibition Test (RFFIT). Although an effective measure to ensure adequate pet rabies vaccination for import purposes, forgeries of the rabies serology reports creates a loophole in this initiative. We, in conjunction with quarantine export/import authorities, have identified a multitude of forged documents including results from samples not tested in our laboratory and reports that have been altered after results were finalized to meet quarantine requirements. Our laboratory provides an online database of all animal serology results tested in our facilities; including official copies of FAVN Reports; accessible by Quarantine Authorities, USDA Veterinarians, and other officials. This study reviews the forgeries detected and practices to improve detection by pet export/import authorities. Increased collaboration of testing facilities and authorities shall heighten future securities of rabies quarantine surveillance and should be considered as an additional resource for destination preservation of rabies free status.

Name: Louis Nel

OP40

Email: louis.nel@rabiesalliance.org

Session: Rabies in Domestic Animals

Country: South Africa

Title: Pan-African Rabies Control Network (PARACON): A unified approach to canine-rabies elimination in Africa

Authors: TP Scott[1], K De Balogh[2], L Taylor[3], A Coetzer[1], N Wright[1], LH Nel[1,3]

Affiliations: [1] University of Pretoria, South Africa; [2] Food and Agricultural Organization, Italy; [3] Global Alliance for Rabies Control, United Kingdom and South Africa

Abstract: Rabies control requires a 'One Health' approach with close coordination between animal and human health sectors across national, regional and continental levels. In Africa, where the per capita death rate of rabies is the highest in the world, such a pan-continental approach to control has been non-existent. In order to attain a coordinated, inclusive and unified network for rabies control across Africa, the Pan-African Rabies Control Network (PARACON) was established in 2014. The primary aim of PARACON is to facilitate and support national programs, encouraging ownership and buy-in from governments and ensuring sustainability and a long-term solution to the control and elimination of rabies. PARACON meetings will take place biennially, and are centered on the One Health approach, with key focal persons from medical and veterinary sectors invited to participate at each meeting. The inaugural PARACON meeting was held in South Africa in June 2015 and was focused around interactive discussion sessions and workshops, whilst publicizing the tools available to aid countries in their efforts towards sustainable rabies intervention strategies. Experts from various global organizations as well as the private sector and other institutions participated in the discussions and shared their experience and expertise during the workshop sessions. The workshops focused on the latest format of the rabies blueprint, which supports various aspects of control and elimination campaigns, including: (1) an educational and advocacy drive; (2) the improvement of surveillance and diagnosis and; (3) the systematic monitoring of progress together with a stepwise planning of the next sequential and logical actions as countries pursue freedom from canine rabies. PARACON will act under its mandate as the unifying umbrella body for rabies elimination in Africa to encourage collaborative efforts among sectors and countries, whilst providing tools and support required for sustainable and effective rabies control and elimination initiatives throughout Africa.

Name: Catherine Filejski

OP41

Email: catherine.filejski@ontario.ca

Session: Rabies in Domestic Animals

Country: Canada

Title: Rabies prevention and control and dog population management strategies in remote northern Ontario First Nation communities

Authors: Catherine Filejski

Affiliations: Ontario Ministry of Health and Long-Term Care, Ontario, Canada

Abstract: The James Bay coastline in northern Ontario has historically served as a conduit for the spread of Arctic Fox rabies from fox populations in Northern Quebec to southern Ontario. Following a large outbreak of Arctic Fox rabies in Northern Quebec in 2011-2012, a puppy on the First Nations reserve of Kashechewan, on Ontario's James Bay coast, was diagnosed with rabies in April 2013. With significant dog over-population issues in the community, and no access to veterinary services or animal rabies vaccinations in this remote, fly-in region of the province, appropriate rabies prevention and control response activities proved extremely difficult in 2013. In November of 2014, the Weeneebayko Area Health Authority, the Ontario Ministry of Health and Long-Term Care and Health Canada announced a two-year partnership with Dogs With No Names and PetSmart Charities of Canada to pilot the use of injectable contraceptives in female dogs to address dog overpopulation and zoonotic disease management issues in the remote and First Nations communities along the James and Hudson Bays coastline of Ontario. Covering the communities of Moosonee, Moose Factory, Fort Albany, Kashechewan, Attawapiskat and Peawanuck, the pilot project launched field operations in June of 2015, microchipping and vaccinating all dogs handled, and injecting intact female dogs with a deslorelin acetate contraceptive implant. 2015 field activities also produced detailed data describing the dog population demographics of each community, which will help in determining cost effective dog population management strategies in the longer term, which are crucial to rabies prevention and control. This presentation will present the interim findings for all six communities covered by the project, give an overview of current ongoing capacity building activities in the communities, and discuss project plans for 2016.

Name: Ryan Wallace

OP42

Email: euk5@cdc.gov

Session: Rabies in Domestic Animals

Country: United States

Title: Evaluation of dog populations and canine rabies vaccination coverage, Uganda 2013

Authors: RM Wallace[1], J Mehal[1], L Adams[1], TJ Nakazawa[1], S Recuenco[2,] B Barnabas[3], M Osinube[1], JD Blanton[1], J Wamala[3]

Affiliations: [1] Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, Atlanta, Georgia, USA; [2] National Institute of Health, Lima, Peru (formerly [1]); [3] Uganda Virology Research Institute, Entebbe, Uganda

Abstract: In Uganda sparse surveillance data exist for the number of human and canine rabies cases, and few studies have captured dog ecology or management information that would be relevant to canine rabies prevention and control. Estimates of dog densities and barriers to canine vaccination are needed for more effective risk modeling assessments and to inform strategies for rabies prevention and control targeting dog populations. In 2013, 798 household surveys were conducted in 24 villages representing five districts in Uganda. Data from the survey consisted of 248 variables that were grouped into the general categories: Household Demographics, Economic Indicators, Dog Demographics, Dog Bite Events, and Rabies Knowledge. An unconditional means model was fit, and the likelihood ratio test was used to evaluate the variation of the response between villages and between districts. Multivariable random intercept models were then fit to evaluate the effects of household- and village-level characteristics on dog ownership and rabies vaccination practices. Survey data showed an average dog-to-human ratio of 1:25; an estimated 1,373,844 owned dogs when extrapolated to the entire country. The multivariable model identified a positive association between dog population and human population density as well as a negative correlation with community poverty level. When adjusting for these variables, the owned dog estimate for Uganda was only 729,486 or 46% lower than conventional dog calculation methods. Modeled canine vaccination rates were also significantly associated with human population density and poverty level, with an estimated national canine rabies coverage of 35%. Dog population estimation methods should consider the influence of poverty on dog ownership. The findings from this study should be validated through field studies to determine the accuracy of the model. If accurate, the methods for dog population estimation could help improve canine rabies vaccination program efficiency and effectiveness.

Name: Emelie Fogelberg

OP43

Email: emeliefogelberg@worldanimalprotection.org

Session: Rabies in Domestic Animals

Country: United Kingdom

Title: Integrating dog population management into rabies elimination programmes around the world

Authors: Emily Mudoga[1], Emelie Fogelberg[2], Ellie Parravani[2]

Affiliations: [1] World Animal Protection, Nairobi, Kenya; [2] World Animal Protection, London, United Kingdom

Abstract: Background: Free-roaming dog populations are considered a problem in many countries for a variety of reasons, including rabies transmission. The misconception that culling dogs will eliminate rabies by reducing the dog population still remains widespread. To combat this misconception, we advocate for a full cycle of DPM, (as set out in the International Companion Animal Management Coalition guidelines), or steps toward this, as an integral part of rabies elimination and in reducing other conflicts between free-roaming dogs and the communities they live in. Objective: To develop humane and sustainable solutions to rabies elimination and in management of free-roaming dogs. Method: World Animal Protection partners with governments around the world, for example in Zanzibar and Bangladesh, to implement humane dog population management programmes as part of their rabies elimination efforts. These programmes can include mass dog rabies vaccination (MDV) campaigns, reproduction control, and education on dog bite prevention and responsible dog ownership. Results: Bangladesh saw a 50% decrease in reported human rabies deaths between 2010 and 2013 following the implementation of DPM efforts in 2011. A 90% decrease is expected to be achieved in 2015. Following introduction of DPM efforts in Zanzibar, a 65% decrease in dog bite incidences and a 100% decrease in reported human rabies deaths was seen between 2008 and 2013. Conclusion: MDV has been proven far more effective than culling in rabies elimination but it is not without challenges. Herd health can be threatened by introduction of unvaccinated dogs, continued culling and dogs not getting vaccinated. What has become clear therefore is that a more holistic approach that looks at the management of dog populations as a whole is the best route to success. This will also lead to a healthier dog population and an improved human-dog relationship.

Name: Miguel Iguala-Vidales

OP44

Email: miiguala@yahoo.com.mx

Session: Wildlife Rabies

Country: Mexico

Title: Rabies Case in a wild animal (lynx) in the State of Sinaloa, and attack on a human

Authors: VM Iguala[1], MM Melo[1], SD Martínez[1], BA Sandoval[1], MI Padilla[1], SM Gómez[1], TR Terán[1], RB Escamilla[1], VI Animas[1], LS Chávez[1], PF Vargas[2], CV Gutiérrez[2], FA Chávez[2], LB Torres[1], PM Vázquez[1], CN Arechiga[1], MI López[1], QA Díaz[1]

Affiliations: [1] Institute of Epidemiological Diagnosis and Reference, SALUD/InDRE, México D.F.; [2] National Center for Preventive Programs and Disease Control, SALUD/CENAPRECE, México D.F.

Abstract: Female patient aged 28, resident of the community of Harrow (close to Choix) attacked on the 4th of June this year. The incident occurred while the patient was in the river of the community, the quoted animal attacks her and while she defends herself gets bitten on her left arm causing a bloody wound, as well as scratches on both legs, and finally she chokes and kills the animal with a stone. On June 5 she is taken by her neighbors to the Integral Hospital of Choix where she received medical care for her wounds in the arm and required 9 stitches; she is then referred to the Health Center of Choix where rabies treatment is begun with the first two dosages of VAH (on the 5th and 8th of June), the attending physician does not indicate if IgAH was implemented. The remains of the Linx are taken to Los Mochis for nervous tissue sampling, the sample is then sent to the InDRE and the results are: DFA positive. Genetically, the virus is grouped in the skunk rabies lineage that is circulating in the northern states of Mexico: Chihuahua, Sonora, Sinaloa and Durango. According to the description of this lineage by Aréchiga et al, in 2010, this virus was associated with the Mustelidae species: Mephitis mephitis in the northern states of Mexico. As additional data this virus is genetically related to a human case in Batopilas, Chihuahua, April 2015.

Name: Brian Bjorklund

OP45

Email: brian.bjorklund@aphis.usda.gov

Session: Wildlife Rabies

Country: United States

Title: A ten-year assessment of raccoon density on Cape Cod, Massachusetts; what have we learned?

Authors: Brian M Bjorklund[1], Ryan JR Bevilacqua[1], Karl von Hone[2], Monte D Chandler[1], Dennis Slate[3], Richard B Chipman[3], Timothy P Algeo[3]

Affiliations: [1] US Department of Agriculture, Wildlife Services, Massachusetts, USA; [2] Division of Natural Resources, Yarmouth, Massachusetts, USA; [3] US Department of Agriculture, Wildlife Services, National Rabies Management Program, Concord, New Hampshire, USA

Abstract: In 2004, raccoon (*Procyon lotor*) rabies was first detected on peninsular Cape Cod and by 2006 had spread its length despite vigorous control efforts by USDA, APHIS, Wildlife Services (WS) and cooperators. In July 2006, USDA Wildlife Services initiated a 10-year project to assess raccoon population trends within densely populated (>549 permanent human residents/km^2 in 2010, swelling 3-fold in summer) South Yarmouth, Massachusetts to help develop strategies for ORV baiting in similar eastern seaboard landscapes. South Yarmouth has seasonal and year-round residential and rental properties, beachfront resorts, numerous restaurants and other businesses, and recreational facilities (e.g., public beaches, mini golf courses, and batting cages). During 2006-2015, 257 unique raccoons were captured on a 3-km^2 study area following the WS National Rabies Management Program density indexing protocol. Annual raccoon densities indexed ranged from 2.9-14.7 raccoons/km^2. Raccoon virus neutralizing antibody (rVNA) levels ranged from 38.9% (2006; n=18) to 93.8% (2012; n=15), but a larger sample size is required in future sampling efforts to have greater confidence in raccoon population antibody levels. Non-target ORV bait uptake represents a loss of resources. During this study, 61 unique striped skunks (*Mephitis mephitis*; density range: 0.0-8.8/km^2), and at least 230 unique Virginia opossums (*Didelphis virginiana*; density range: 2.3-15.0/km^2) were captured and released. Trends in raccoon, skunk, and opossum density indices vary, perhaps in response to several factors, suggesting a complex rabies control environment. Further complexities may be expected and have been experienced here where human populations are high. While important lessons have been learned on the deployment of raccoon rabies control strategies, areas with a broad human-wildlife interface that occur on much of Cape Cod present many challenges to be addressed to better ensure success in rabies control and elimination that may also be applied in similar areas along the eastern seaboard of the U.S.

Name: Silvana Favoretto

OP46

Email: srfavoretto@usp.br

Session: Wildlife Rabies

Country: Brazil

Title: Identification of a distinct genetic variant of rabies virus probably maintained by canid populations in Paraguay

Authors: Júlio César Augusto Pompei[1], Danielle Bastos Araujo[2], Fátima Aideé Vázquez, Rosa Alicia Galeano, Luz Marina Delgado, Ramón Recalde, Keila Iamamoto[3], Luzia Fátima Alves Martorelli[4], Mônica Martini[3], Rodrigo Garcia, Dalva Assis, Otorino Cosivi[3], Aurélio César Fiori Amarilla, Edison Luis Durigon[2], Silvana Regina Favoretto[3]

Affiliations: [1] PANAFTOSA, Panamerican Health Organization/World Health Organization (PAHO/WHO), Duque de Caxias, Rio de Janeiro, Brazil; [2] University of Sao Paulo, Laboratory of Clinical and Molecular Virology, Center for Research on Rabies, Sao Paulo, SP, Brazil; [3] Pasteur Institute of São Paulo, Brazil; [4] Center of Zoonosis Control, São Paulo, SP, Brazil

Abstract: Paraguay has no register of human cases of rabies since 2004 and the last case in dogs was reported in 2009 due to the variant maintained by the common vampire bat (*Desmodus rotundus*). In 2014 a dog from the Loma Plata District was diagnosed as positive with report of aggression to a boy. All the required measures of control were successfully adopted: vaccination door to door of cats and dogs, vaccination of bovines in the focus and perifocus (05 Km), active search of cases and post-exposure treatment to the boy and contacts. The epidemiological investigation revealed that the dog was not vaccinated and probably attacked by a crab eating fox – "zorro" (*Cerdocyon thous*). The sample was diagnosed by the Official Veterinary Service of the Country (SENACSA) and sent to the Center on Rabies Research from the University of São Paulo, Brazil for antigenic and genetic characterization. The antigenic profile was compatible with the variant maintained by dogs and the genetic characterization showed that the sample segregated in the canine (domestic and wild species) related group, but in an independent subgroup not related to any previously isolated samples when compared with canine samples from Paraguay (Between group mean distance: 5.3%) and other countries from Latin America (8.8% to 10.9%). These genetic results are indicative that rabies virus is circulating in the "zorros" adapted in a different variant. The variant maintained by the crab eating fox – related to the maintained by dogs - is well characterized and studied in the Northeast of Brazil. Our results are evidence that even with the control of rabies in domestic animals; the virus still circulates in wildlife, demonstrating the importance of continuous and improved activities of surveillance and control of the disease in wild species in order to prevent cases in domestic animals and humans.

Name: Karsten Hueffer

OP47

Email: khueffer@alaska.edu

Session: Wildlife Rabies

Country: United States

Title: Rabies ecology in Alaska, challenges and opportunities

Authors: Karsten Hueffer

Affiliations: University of Alaska Fairbanks, USA

Abstract: Alaska, the largest State of the US with sparse population and extremely remote areas faces many health challenges, including rabies in wildlife. Rabies is prevalent in coastal regions of Alaska and absent in the interior. The understanding of the dynamics is limited. Rabies vaccination of dogs in many endemic areas in remote Alaska is limited. Several entities engage in research and management of rabies in Alaska. Public health agencies focus on investigation of human exposures to determine the appropriateness of post exposure prophylaxis and improvement of dog vaccination; game manager are involved in surveillance of wildlife, while the academic institutions try to elucidate fundamental dynamics of rabies ecology and pathogenesis of this important disease in the North. A dramatically changing climate introduces additional complexity. I will discuss the intersection of these efforts and how the different stakeholders can support each other's missions and approaches.

Name: Kim Pepin

OP48

Email: kim.m.pepin@aphis.usda.gov

Session: Wildlife Rabies

Country: United States

Title: Spatial spread of skunk rabies in Northern Colorado, USA

Authors: Kim Pepin, Amy Davis, Justin Fisher, Amy Gilbert

Affiliations: US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA

Abstract: The South Central Skunk (SCSK) variant of rabies virus (RABV) has been expanding in distribution in the US, leading to incursions of skunk rabies in areas that were previously free of carnivore rabies. Colorado has experienced several incursions of the SCSK variant from neighboring states where the variant is enzootic in striped skunk (*Mephitis mephitis*) populations. Two epizootics began in northern Colorado in 2012, and have been spreading geographically during the last 3 years. We used an occupancy model to jointly estimate prevalence of RABV through time and the effects of different invasion parameters, specifically persistence, initial colonization and recolonization. We quantified the effects of spatial proximity to sites with infected skunks on invasion parameters, relative to the effects of long-distance movement of RABV. The model is based on passive surveillance of skunks reported by the public to county or state public health departments. In addition to prevalence and invasion parameters, we also estimated an effect of human population size on reporting. We found that prevalence showed strong seasonality and was considerably higher than the colonization rates. Both initial colonization and recolonization depended strongly on the presence of RABV nearby, but both were rare overall at 8 km increments. Our model suggests that the epizootic is moving south with decreasing prevalence over time.

Name: Efren Jaramillo-Reyna

OP49

Email: ejrvirus@gmail.com

Session: Wildlife Rabies

Country: Mexico

Title: Rabies in aggressor carnivores of Nuevo Leon, Mexico

Authors: E Jaramillo-Reyna[1], JZ Villarreal-Pérez[1], FJ Garcia-Elizondo[1], MJ Zamudio-Ozuna[1], EC García-García, MA Zúñiga-Ramos[2], N Aréchiga-Ceballos[3]

Affiliations: [1] State Laboratory of Public Health, Ministry of Health of Nuevo León, México; [2] School of Biological Sciences, University of Nuevo León, México; [3] Rabies Laboratory, Institute of Epidemiological Diagnosis and Reference, México

Abstract: In Mexico human rabies transmitted by dogs is absent since 2006, reflecting a success of the rabies virus (RV) vaccination campaign in the country. By contrast, wild-living mammals have revealed as the principal source of transmission of rabies (RV) to humans. Nowadays in the border between Mexico-USA several rabies cases on wild-living and domestic animals have been identified. One of the most important was the canine case, a domestic dog infected with a dog-coyote rabies virus antigenic variant (RABV). Other cases have been documented in cats, skunks and foxes. Nevertheless, all of them are related to skunk RABV. In Nuevo Leon State during 2008 and 2009, three cases of rabies in wild-living mammals were reported: 2 in skunks (*Mephitis macroura*) with history of human attacks and one fox (*Urocyon cinereoargenteus*), that have attacked a male. Considering the foregoing, the aim of this study was to determine the genetic and phylogenetic relationship of the RABV isolated in Nuevo Leon. Brain samples were obtained from the skunks and the fox. Using the fluorescent antigen test (FAT), antigenic characterization, RT-PCR and nucleotide sequencing of the semi-variable region of the nucleoprotein gene. Both skunks were characterized as RABV 8 and the fox was RABV 7. The sequencing showed that RV isolated from the skunks are closely related to RABV circulating in the State of San Luis Potosi, but also grouped with RABV of Zacatecas and Chihuahua. Finally, the RABV of the fox was placed on the lineage pertaining of foxes in the Mexico-USA border. These results reveals circulation RABVs in carnivores with a history of aggression and human contact. It is required to increase the RV surveillance on the border between the two countries through the North America Rabies Management Plan (NARMP).

Name: Timothy P Algeo

OP50

Email: Timothy.P.Algeo@aphis.usda.gov

Session: Wildlife Rabies

Country: United States

Title: Using raccoon (*Procyon lotor*) density data and remotely-sensed landscape information to develop a habitat connectivity-based rabies risk model

Authors: TP Algeo[1], M Palace[2], D Slate[1], RM Caron[2], T Atwood[3], S Recuenco[4], RB Chipman[1], MJ Ducey[2]

Affiliations: [1] US Department of Agriculture, Wildlife Services, National Rabies Management Program, Concord, New Hampshire, USA; [2] University of New Hampshire, Durham, New Hampshire, USA; [3] United States Geological Survey, Alaska Science Center, Anchorage, Alaska, USA; [4] National Institute of Health, Lima, Peru

Abstract: The USDA, APHIS, Wildlife Services National Rabies Management Program (NRMP) and its cooperators have been conducting oral rabies vaccination campaigns (ORV) to prevent the spread of the raccoon (*Procyon lotor*) variant of rabies into mid-western U.S. states and Canada since 1997. Raccoon density index (RDI) information is critical to ORV planning and these have ranged from 0 to >25 raccoons/km^2. Efforts to eliminate raccoon rabies will be conducted in part on landscapes comprised of pine (*Pinus* spp.)-dominated forests, and RDI on those landscapes are among the lowest observed. The NRMP conducted 15 RDI studies in 11 locations in 6 eastern states during 2007-2012 with an RDI that ranged from <1-3.3 raccoons/km^2. Of seven a priori candidate AICc models considered for understanding raccoon use of pine-dominated landscapes, the model that included only habitat edge (Akaike wi = 0.58) was top-ranked. Raccoon presence (capture/collection) data (n = 2,896) during 2012-2014 were then analyzed via a Maximum Entropy (Maxent) procedure to assess raccoon habitat suitability across broader pine-dominated landscapes in Massachusetts, Florida, and Alabama, taking into consideration landscape features likely to moderate the effect from pine landscapes such as elevation, soils, temperature, precipitation, distance to water and roads, buildings, and human population. Resulting Maxent models varied among states and suggested that the most influential variables in terms of raccoon distribution were precipitation (MA), temperature (FL), and soils (AL). Finally, Maxent probability surface maps were ingested into Circuitscape, which provided conductance visualizations as aids to identifying potential areas of connectivity for raccoons, and hence, risk for the movement of rabies. These results suggest opportunities for capitalizing on the relatively low RDI of pine-dominated regions with minimized risk for rabies perpetuation and spread, which could result in significant cost-savings.

Name: Amy Gilbert

OP51

Email: amy.t.gilbert@aphis.usda.gov

Session: Oral Rabies Vaccine and Rabies Control

Country: United States

Title: Efficacy of variable dose ONRAB delivery in striped skunks (Mephitis mephitis)

Authors: Amy Gilbert[1], Shylo Johnson[1], Scott Bentham[2], Alex Beath[2], Kurt VerCauteren[1]

Affiliations: [1] US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA; [2] Artemis Technologies Inc., Guelph, Ontario, Canada

Abstract: ONRAB vaccine has been successfully used to control circulation of raccoon and arctic fox variants of rabies virus in raccoons, skunks and foxes in Canada. In the US, skunks are a reservoir host of three rabies virus variants and an important spillover host associated with the raccoon rabies virus variant. Though immunogenicity and efficacy studies evaluating ONRAB Ultralite baits (ULB) have been conducted with captive striped skunks (Mephitis mephitis) in Canada with positive results, research at the USDA/APHIS/Wildlife Services National Wildlife Research Center with captive skunks revealed suboptimal interaction and consumption of ULB, suggesting a potential need to reduce bait size for improved oral manipulation and contact with vaccine. To support development of this idea, we tested varying volumes of ONRAB by direct installation into the oral cavity (DIOC) of skunks (3 groups of n=5). For comparison, we also offered an egg flavored ULB to one group (n=5) and another group (n=5) received culture media by DIOC. Rabies virus neutralizing antibody titers (rVNA) were evaluated at regular intervals post vaccination (pv) and all skunks were inoculated with 0.5ml of a dog strain of rabies virus into each masseter muscle on day 335 pv. Skunks were observed for 75 days post challenge. Five control skunks that received culture media DIOC, and one skunk that did not consume a ULB during the vaccination phase, succumbed to rabies infection. In the DIOC vaccination groups (n=15), one skunk did not seroconvert nor survive challenge and one nonspecific death occurred on day 293, but 13 skunks had high levels of rVNA pv and survived challenge. Among four skunks that consumed ULB, none seroconverted pv and 50% succumbed to challenge. The results suggest it may be possible to reduce the volume of ONRAB contained in baits without compromising efficacy, to facilitate development of smaller baits.

Name: Monte Chandler

OP52

Email: monte.d.chandler@aphis.usda.gov

Session: Oral Rabies Vaccine and Rabies Control

Country: United States

Title: The Cape Cod oral rabies vaccination program – where are we now?

Authors: Monte D Chandler[1], Brian M Bjorklund[1], Ryan JR Bevilacqua[1], Timothy P Algeo[2], Dennis Slate[2], Karl W von Hone[3], Amy L von Hone[4], Erika Woods[5], Richard B Chipman[2]

Affiliations: [1] US Department of Agriculture, Wildlife Services, Massachusetts, USA; [2] US Department of Agriculture, Wildlife Services, National Rabies Management Program, Concord, New Hampshire, USA; [3] Division of Natural Resources, Yarmouth, Massachusetts, USA; [4] Yarmouth Health Department, Yarmouth, Massachusetts, USA; [5] Barnstable County Department of Public Health and Environment, Barnstable, Massachusetts, USA

Abstract: Raccoon (*Procyon lotor*) variant rabies was first detected on peninsular Cape Cod, MA during the spring of 2004, despite ten years of cooperative control efforts. USDA, APHIS, Wildlife Services began full-time involvement in 2001. By 2006, raccoon-variant rabies was detected at Cape Cod's tip, and efforts since then were aimed at reducing rabies cases while working towards elimination. Carefully timed and planned strategic oral rabies vaccine (ORV) barrier movements toward the mainland based on rabies surveillance results occurred during the Fall campaigns of 2010, 2013, and 2015. Rabies cases on Cape Cod have decreased significantly since the initial outbreak (n=124-160 during the first two years of the epizootic), to fewer than 11 annually over a geographically shrinking area from 2007-2015. In response to funding challenges, and the difficulty of distributing ORV baits in areas with high human densities, ORV bait stations were used experimentally from 2006-2008 and operationally from 2009-2015 as an integral part of ORV operations. The Fall 2013-Spring 2015 ORV zone included 242 ORV bait stations in both high (1.8 bait stations/km^2) and low (0.1 bait stations/km^2) density strategies. The proposed Fall 2015 ORV zone will feature 319 bait stations and hand-baiting over approximately 615 km^2 in an area which hasn't been baited since the initial epizootic in 2004, including areas up to the Canal separating Cape Cod from the mainland. Serological response within the coated sachet hand-baiting zone was 40.6% (n=165), and 52.1%, (n=140) in the high density bait station subset. From this point, the five year goal of the Cape Cod Oral Rabies Vaccination Program is the elimination of raccoon-variant rabies from the peninsula and the re-establishment of a sufficient mainland barrier utilizing bait stations with the goal of preventing the re-emergence of terrestrial rabies on Cape Cod.

Name: Chad Blass

OP53

Email: chad.r.blass@aphis.usda.gov

Session: Oral Rabies Vaccine and Rabies Control

Country: United States

Title: Skunks' movements and flight lines for oral rabies vaccination

Authors: Shylo Johnson[1], Sam Mills[2], Chad Blass[1], Travis Flanagan[2], Nikki Crider[1], Justin Fischer[1], Kurt VerCauteren[1]

Affiliations: [1] US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA; [2] US Department of Agriculture, Wildlife Services, West Virginia, USA

Abstract: Raccoons (*Procyon lotor*), skunks (primarily striped skunks [*Mephitis mephitis*]), foxes (primarily gray foxes [*Urocyon cinereoargenteus*]), and coyotes (Canis latrans) are all terrestrial host species for rabies virus variants in the continental United States. Successful elimination of terrestrial rabies requires that oral rabies vaccination (ORV) programs are able to target multiple host species and adjust ORV planning to account for multiple target species where these species overlap. For raccoon ORV, the standard bait density is 75 baits/km^2 with aircraft flight lines at 750 m apart. Skunks are a common spillover host in areas where the raccoon variant is enzootic and current ORV efforts exist for targeting raccoons. Achieving elimination may also require targeting skunks. Using data we gathered on skunk movements, we will compare standard bait density and flight lines to high bait density and flight lines to evaluate possible bait uptake by skunks. We collected movement data from nine GPS collared skunks that had been exposed to 300 baits/km^2 with 250 m flight line spacing. The mean pre- and post-bait drop core home ranges were 5.2 ha (SE 1.21 ha) and 5.28 ha (SE 1.28 ha), respectively, and the mean pre- and post-bait drop overall home ranges were 26.16 ha (SE 6.24) and 27.46 ha (SE 5.54), respectively. We will use this information to estimate and compare potential number of baits dropped within and number of flight lines crossing the core and overall space use areas for the two different flight line spacing and bait densities. Implications of the higher bait density and tighter flight lines for eliminating raccoon variant in skunks will be discussed.

Name: Emmanuelle Robardet

OP54

Email: emmanuelle.robardet@anses.fr

Session: Oral Rabies Vaccine and Rabies Control

Country: France

Title: Control and elimination of rabies in the Baltic States

Authors: Emmanuelle Robardet[1], Evelyne Picard-Meyer[1], Marianna Dobroštana[2], Ingrida Jaceviciene[3,8], Katrin Mähar[4], Zita Muižniece[2], Gediminas Pridotkas[3], Marius Masiulis[5], Enel Niin[6], Edvīns Olševskis[2,7], Florence Cliquet[1]

Affiliations: [1] ANSES, Nancy Laboratory for Rabies and Wildlife, Malzéville, France; [2] BIOR, Institute of Food Safety, Animal Health and Environment, Riga, Latvia; [3] National Food and Veterinary Risk Assessment Institute of Lithuania, Vilnius, Lithuania; [4] Estonian Veterinary and Food Laboratory, Tartu, Estonia; [5] State Food and Veterinary Service, Vilnius, Lithuania; [6] Estonian Veterinary and Food Board, Tallin, Estonia; [7] Food and Veterinary Service, Riga, Latvia; [8] Vilniaus Kolegija/University of Applied Sciences Faculty of Agrotechnologies, Vilnius district, Lithuania

Abstract: Rabies is a fatal zoonosis that still causes nearly 70,000 human deaths every year. In Europe, the Oral Rabies Vaccination (ORV) of red foxes (*Vulpes vulpes*) was developed in the 1980s and has demonstrated its effectiveness in the eradication of the disease in Western and some Central European countries. Following the accession of the three Baltic countries - Estonia, Latvia and Lithuania - to the European Union in 2004, subsequent financial support has allowed the implementation of regular ORV campaigns since 2005-2006. This paper reviews 10 years of surveillance efforts and ORV campaigns in these countries resulting in the nearly eradication of the disease. The various factors that may have influenced the results of monitoring were assessed using generalized linear models on bait uptake and immunisation rate. As shown in previous studies, juveniles have lower bait uptake level than adults. For the first time, raccoon dogs (*Nyctereutes procyonoides*) were shown to have significantly lower bait uptake rate compared with red foxes. This result suggests potentially altered ORV effectiveness in this invasive species compared to the red foxes. An extensive phylogenetic analysis demonstrated that the North-East European group (NEE) of rabies viruses is endemic in all three Baltic countries. Although successive oral vaccination campaigns have substantially reduced the number of detected rabies cases, sporadic detection of the C lineage underlines the risk of reintroduction via westward spread from bordering countries. Vaccine induced cases were also reported for the first time in non-target species (*Martes martes* and *Meles meles*).

Name: Conrad Freuling

OP55

Email: Conrad.Freuling@fli.bund.de

Session: Oral Rabies Vaccine and Rabies Control

Country: Germany

Title: Validation of a challenge model for red foxes – what is the best parameter to express infectivity?

Authors: Conrad Freuling[1], Elisa Eggerbauer[1], Stefan Finke[1], Christiane Kaiser[3], Christian Kaiser[3], Antje Kretzschmar[3], Elke Lange[2], Thomas Mettenleiter[1], Tobias Nolden[1], Steffen Ortmann[3], Peter Schuster[3], Charlotte Schröder[2], Jens Teifke[2], Ad Vos[3], Thomas Müller[1]

Affiliations: [1] Friedrich-Loeffler-Institut (FLI), Institute of Molecular Virology and Cell Biology, Greifswald-Insel Riems, Germany; [2] FLI, Department of Animal Husbandry and Biorisk Management; [3] IDT Biologika GmbH, Dessau-Rosslau, Germany

Abstract: Licensing of vaccines against infectious agents requires the establishment and validation of an appropriate challenge model. For oral rabies vaccines targeting the red fox (*Vulpes vulpes*) a novel challenge virus was generated and validated in foxes. Initially isolated from a rabid fox brain this virus (named Fox Krefeld 148) was passaged in MNA cells until reaching a titre of 106.05 TCID50/ml (105,4FFU/ml) and sequenced to verify the origin of the virus. When tested in mice this virus suspension had a titre of 106.7 MICLD50/ml. To establish a valid challenge model, foxes were infected intramuscularly (M. masseter) with various dilutions 1:100, 1:1000, 1:10000, 1:25000 and 1:50000. All challenged animals died and only the incubation period increased slightly from 10 dpi to 14 dpi. To take it to an extreme, the virus was then diluted 1:1000000, a calculated titre 100.7 MICLD50/ml. Even with this high dilution all inoculated foxes had died by day 31 pi (mean 22.8 dpi). The inoculum was retested for infectivity in MNA cells, but viable virus could not be detected. Also, three runs of real-time PCRs of independently extracted RNA revealed two negative and one weak positive result. In summary, we used a virus suspension that was negative in cell culture, weak positive or questionable in realtime RT-PCR, that only killed 1 out of 10 mice, but was highly potent in the homologous reservoir, the red fox. These results do not only question the diagnostic sensitivity but also highlight the differences in various test systems used for infectivity.

Name: Are Berentsen

OP56

Email: Are.R.Berentsen@aphis.usda.gov

Session: Oral Rabies Vaccine and Rabies Control

Country: United States

Title: Mongoose research by USDA Wildlife Services in Puerto Rico, 2011 – 2015: implications for oral rabies vaccination

Authors: Are R Berentsen[1], Shylo R Johnson[1], Kurt C VerCauteren[1], Ken Gruver[2], Frank Boyd[2], Amy T Gilbert[1], Richard B Chipman[3]

Affiliations: [1] US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA; [2] US Department of Agriculture, Wildlife Services, Alabama/Puerto Rico, USA; [3] US Department of Agriculture, Wildlife Services, National Rabies Management Program, Concord, New Hampshire, USA

Abstract: The small Indian mongoose (*Herpestes auropunctatus*) was introduced throughout the Caribbean in the late 19th century to control rat (*Rattus* spp.) damage to sugar cane. Mongooses failed at suppressing rats and are now considered a pest species. In addition, they are a rabies reservoir on several islands, including Puerto Rico, where mongooses account for up to 70% of animals testing positive for rabies annually. The last human rabies fatality associated with the mongoose lineage was in 2003, and approximately 287 mongoose bites are reported each year. There is no oral rabies vaccine licensed for use in mongooses although experimental vaccines have shown promise. In pursuit of an oral rabies vaccination (ORV) program for mongooses in Puerto Rico, USDA Wildlife Services has conducted a series of studies including bait flavor preference, population density, sero-surveys and home range estimates. Bait flavor studies suggest mongoose preferred cheese-flavored baits over fish and coconut although egg flavor was preferred over unflavored baits. Population densities range from 45-75 mongooses/km^2. Up to 40% of 112 mongooses sampled during 2011-2014 were positive for rabies-virus neutralizing antibodies. Preliminary Minimum Convex Polygon home range estimates range from 0.1-1.57 km^2. Potential future studies include non-target population density estimates and biomarker studies to evaluate bait uptake by mongooses. A more thorough understanding of mongoose ecology is essential in designing an ORV program for mongooses in Puerto Rico.

Name: Guillermo Hernandez Mejia

OP57

Email: rabiacefpphgo@hotmail.com

Session: Bats and Lyssavirus: Host and Virus Diversity

Country: Mexico

Title: Adaptation of the hematophagous bat (*Desmodus rotundus*) to climatic and demographic changes in the state of Hidalgo

Authors: Guillermo Hernández Mejia[1], Adalberto Morales Fragoso, Eduardo Saldaña Romero, Anahel Carpio Ruano

Affiliations: [1] Hidalgo State Committee of Animal Health, Mexico

Abstract: The hematophagous bats have been presenting modifications in their ecological conditions for survival as are light tolerance and survival at high altitudes. The bats prefer a climate with a relative constant temperature and humidity throughout the year, close to 22^{0}C and 45% humidity. They do not tolerate profound modifications. They can be found from sea level, to more than 2000 AMSL. In the State of Hidalgo, within the epidemiological actions concerning the Bovine Paralytic Rabies campaigns, procedures are held to capture hematophagous bats derived from the monitoring operations, the presence of bats has been reported in climatic and demographic conditions that surpass their normal survival habits, these conditions vary from 2400 to 2600 AMSL, with temperatures up to 46^{0}C and humidity levels as far as 90%. In the table are shown data from the reports of the hematophagous bat in municipalities where the climatic and demographic conditions pas the survival parameters for this specie. In the year 2014 and 2015 the following captures involving hematophagous bats are reported.

Year	Municipality	Locality	Type of capture	Altitude	Relative humidity	Month of capture
2014	Zimapan	Camposanto del oro	Corral	2570	80%	March
	Mineral del Chico	El Puente	Corral	2616	85%	June
	Alfajayucan	La Salitrera	Refuge	2456	70%	August
	Nopala	Dañu	Corral	2487	75%	November
	Nopala	Manguini	Corral	2576	75%	November
2015	El Arenal	San Jerónimo	Corral	2623	75%	January
	Calnali	Coyula	Corral	3556	90%	February
	Nopala	Loma del Toro	Corral	2424	80%	June

In the year 1998 in the State of Hidalgo, epidemiological actions were begun related to the Bovine Paralytic Rabies Campaign in 45 enzootic municipalities, currently the number of municipalities has grown due to the presence of hematophagous bats in 59 of the 84 municipalities in the state of Hidalgo. All this because of the reports on the presences of new hematophagous bat refuges, the consequent aggression to susceptible animals and new positive cases of rabies transmitted by hematophagous bat bite.

Name: Alejandro Zaldivar Gomez

OP58

Email: al3xvet@gmail.com

Session: Bats and Lyssavirus: Host and Virus Diversity

Country: Mexico

Title: Environmental niche modelling of common vampire bats in the mexican plateau: new insights into the macroecological limiting factors

Authors: A Zaldivar-Gomez[1], ME Jaimes-Benitez[2], CG Perez-Colin[2], JA Casillas-Aguilar[3], JM Jimenez-Estrada[1], C Delgado-Urbina[1]

Affiliations: [1] State Public Health Laboratory of the State of Mexico, Toluca, Mexico; [2] Committee of Farming Protection of the State of Mexico, Toluca, Mexico; [3] State Public Health Laboratory of the State of Morelos, Cuernavaca, Mexico

Abstract: The common vampire bats (*Desmodus rotundus*) is the cause of large economic losses to livestock holdings in Latin America through decreases in milk production, weight gain and increased risk of infection by rabies. Vampire bats distribution tends to be limited to the tropics at lower elevations, at latitudes beyond the position at present of the 10°C minimal isotherm for January. The study area is located in the southern part of the Mexican plateau, which covers the State of Mexico, Morelos, Mexico City, and northern part of Guerrero. The climate is characterized by a transition in temperature and precipitation between two bioclimatic regions; this has led to an absence of vampire bat towards the north due to adverse natural conditions. Data was obtained indicating vampire bat presence in the region at 264 georeferenced points. A set of high-resolution environmental layers were built to generate potential distribution maps and were projected to WGS 84 UTM 14N. The maps of temperature and precipitation were interpolated through a multivariate geostatistical model (cockriging) and were used to calculate 19 bioclimatic variables, moreover, density of cattle, elevation and slope. Through a Principal Component Analysis, the set of 22 original variables was transformed into five PC's with 95% of the variance explained. The Maxent application was used to produce the potential distribution model. The results indicated highly suitable habitat for *D. rotundus* occurs troughout most of tropical climate into the south of Mexican plateau. The relative contribution of environmental predictors, represented by the orthogonal arrangement of PC's were: PC1, PC3 and PC2; that explain 85% of total variance registered in the original variable, and which can be explained by annual regional differences in temperature, density of cattle and precipitation, respectively. These environmental conditions have a direct effect on the marginalization of their distribution and abundance.

Name: David Moran (Presented by Amy Gilbert)

OP59

Email: dmoran@ces.uvg.edu.gt (amy.t.gilbert@aphis.usda.gov)

Session: Bats and Lyssavirus: Host and Virus Diversity

Country: Guatemala

Title: Home range, foraging fidelity and feeding behavior of the common vampire bat (Desmodus rotundus) in an agricultural landscape in Guatemala

Authors: David Moran[1], Danilo Alvarez[1], Kevin Olival[2], Amy Gilbert[3]

Affiliations: [1] Center for Health Studies at University of the Valley of Guatemala; [2] EcoHealth Alliance, New York, New York, USA; [3] US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA

Abstract: Common vampire bats (*Desmodus rotundus*) are highly social and abundant, and frequently come into contact with domestic animals, primarily cattle. Given its unique feeding and social behaviors, *D. rotundus* may act as a vector of infectious diseases. Although *D. rotundus* is recognized as the principal reservoir host of rabies in Latin America, very little is known of its ecology, including distances traveled between roosting locations and foraging habitats. The objectives of this study were to characterize the home range and foraging patterns of *D. rotundus* in an agricultural landscape. Radio transmitters were attached to a total of 30 individual bats in an agricultural zone of southern Guatemala, in proximity to cattle farms, during three separate years. Monitoring was performed by teams equipped with hand-held antennas and receivers during the day using walking trails and during the night at fixed stations. We observed 70% of monitored bats returned to the same cattle herds to feed nightly, despite abundant local availability of other cattle farms. Four individuals were detected feeding while cows were resting in a pasture at night. These bats fed until dawn and we were able to track them to a cave located one kilometer from the cattle pasture. Bats returned to the same areas to forage nightly, except for the week of the full moon during which individuals did not leave the roost. Our results show that vampire bat foraging within agricultural landscapes is locally stable in Guatemala, yet no bats were recaptured across the different years of study. Thus, over longer time scales there is turnover of local populations, perhaps due to migration or mortality or both. Knowledge of vampire bat ecology is useful for surveillance, prevention and control activities during rabies outbreak investigations.

Name: Lorraine McElhinney

OP61

Email: lorraine.mcelhinney@apha.gsi.gov.uk

Session: Bats and Lyssavirus: Host and Virus Diversity

Country: United Kingdom

Title: Detection and diversity of European Bat Lyssaviruses

Authors: LM McElhinney[1,2], DA Marston[1], C Freuling[3], T Müller[3], AR Fooks[1,2]

Affiliations: [1] Animal and Plant Health Agency (APHA), Weybridge, United Kingdom; [2] NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, University of Liverpool, United Kingdom; [3] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald - Insel Riems, Germany

Abstract: The first rabies infected insectivorous bat in Europe was recorded in Hamburg, Germany, in 1954. Since surveillance records began in 1977, 1098 cases of bat rabies have been reported to WHO Rabies Bulletin Europe. Prior to the death of a bat worker in Finland in 1985, very few bat rabies cases were reported. But enhanced surveillance in the two subsequent years (1986-87), identified 276 cases (24% of all reported cases to date). The vast majority (>97%) of bat rabies cases in Europe are attributed to European Bat Lyssavirus Type-1 (EBLV-1) infected serotine bats (*Eptesicus serotinus*). A much smaller number (<30 cases) are associated with European Bat Lyssavirus Type 2. Unlike EBLV-1, which has been detected during active surveillance in bat species other than Serotine bats, the chiropteran host range of EBLV-2 is presently restricted to just two bat species *M. daubentonii* and *M. dascyneme*. *M. dasycneme* is confined to Central and Eastern Europe, whereas *M. daubentonii* is widely distributed throughout Europe. In addition to EBLV, insectivorous bats in Europe have also been associated with other lyssaviruses namely Bokeloh Bat Lyssavirus, West Caucasian Bat Lyssavirus and Lleida Bat Lyssavirus, although only 1-2 cases have been reported to date. The relatively small number of EBLV-2 cases in Europe prompted the establishment of a network of European labs (Germany, Switzerland, Netherlands, Finland, France and UK) to collate all available viruses and data. Despite the relatively low number of EBLV-2 cases, a surprisingly large amount of anomalous data had been published in the scientific literature and Genbank, which we have since collated and clarified. Partial N gene sequence data for EBLV-2, defined both geographical and host specific relationships but no chronological clustering was evident. Full genome sequence has now been obtained for all available isolates (n=26) and will further our understanding of the diversity and molecular evolution of EBLV-2 in Europe.

Name: Cuc Tran

OP62

Email: euk5@cdc.gov

Session: Epidemiology and Modeling

Country: United States

Title: Estimation of human and canine rabies deaths in Haiti utilizing country-specific data; a comparison of three estimation methods

Authors: C Tran[1], JD Blanton[1], K Hampson[2], N Fenelon[3], L Orciari[1], M Etheart[1], A Destine[3], K Crowdis[1], C Francisco[4], F Ludder[4], M Millien[4], RM Wallace[1]

Affiliations: [1] Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, Atlanta, Georgia, USA; [2] University of Glasgow, United Kingdom; [3] Ministry of Public Health and Population, Department of Epidemiology and Laboratory Resources, Haiti; [4] Ministry of Agriculture and Natural Resources and Rural Development, Department of Animal Health, Haiti

Abstract: The Republic of Haiti is one of a few countries in the Western Hemisphere where canine rabies is still endemic. Estimation methods have predicted over 100 human deaths each year, yet existing surveillance mechanisms have detected few of these cases. Global data has been used for prediction models in most developing countries due to the lack of country-specific data required for accurate estimation. In 2013, a passive animal rabies surveillance program was developed in Haiti which identified 66 laboratory-confirmed rabid animals and 36 probable cases in a two year period compared to just 4 cases diagnosed in the 2 years prior to the surveillance program. In 2014 more than 2,000 KAP surveys were conducted amongst dog owners attending a mass vaccination clinic. Among these respondents, 14.7% reported owning a dog that had died of rabies-like illness in the past year and 3.4% had experienced a dog bite in the year preceding the survey. These studies present the first Haiti-specific measures for developing rabies burden prediction models. Three methods were used to estimate the human rabies burden, utilizing these data: simple linear calculation, economic modelling, and a probability decision-tree model. Results estimated 550, 250, and 399 human rabies deaths, respectively. Haiti currently reports an average of 7-14 human rabies deaths each year, far below what has been estimated by all modeling methods. Current diagnostic and surveillance infrastructure in Haiti is not sufficient to accurately detect human rabies cases. Regardless of estimation methods utilized, all predict high rates of human rabies deaths, comparable to those seen in high burden rabies countries such as Bhutan and Kenya. Domestic and international attention should be focused on primary prevention efforts such as mass canine rabies vaccination, and secondary efforts that include more efficient distribution and utilization of human rabies vaccine.

Name: Yanislet Cordero Aguliar

OP63

Email: ycordero@ipk.sld.cu

Session: Epidemiology and Modeling

Country: Cuba

Title: Epidemiology and molecular characterization of rabies in Cuba from 2003 to 2014

Authors: Yanislet Cordero Aguilar[1], Maria de los Angeles Ribas Antúnez[1], Helena Beatriz Carvalho[2], Yuliet Cintra Ramirez[1], Yahisel Tejero[1], Jusaima González[1], Daileny Pérez[1], Juliana Castilho[2]

Affiliations: [1] Pedro Kouri Institute of Tropical Medicine, Department of Virology, National Rabies Reference Laboratory, Ministry of Public Health, Havana, Cuba; [2] Pasteur Institute of Sao Paulo, SP, Brazil

Abstract: Introduction: Rabies is a zoonotic disease with high incidence worldwide. In Cuba urban rabies remains a significant problem with high levels of infection. Therefore, knowledge of its epidemiology is of great importance for the control of the disease. Objective: This study was conducted in order to determine the epidemiological and molecular characteristics of rabies virus in the country. Methods: We studied 2598 samples from animal's brain tissue around the country in the period 2003-2014. More than 15 species were under surveillance. They were tested by direct immunofluorescence and RT-PCR assays. N gene sequencing and phylogenetic analysis were performed in selected 27 positive samples. Results: We found virus circulation throughout the country with a positivity that ranged from 93.2% to 18.8%. Central and western regions were the most affected with 60% and 47.9% respectively. Mongoose remained as the main reservoir of the virus, showing the highest rates of positivity (66.4%). It was evident a high relationship between a history of aggressive behaviour and positive results to infection (53.4%) principally in the cats (64.6%). The five bats that tested positive were from the *Artibeus jamaicensis* species. Phylogenetic analysis showed the presence of genotype I. All variants that presently circulate in Cuba are derived from a common ancestral. Terrestrial strains belonged to two different clusters (A and B). In A were grouped the species from the West and East of the country while B were grouped those from the East and Center. Thus, there was a tendency to cluster by regions regardless of the species analyzed. Conclusion: This study showed a high prevalence of rabies in Cuba, other research is needed to improve the surveillance and control of rabies.

Name: Meggan Craft

OP64

Email: craft004@umn.edu

Session: Epidemiology and Modeling

Country: United States

Title: Raccoon contact networks and rabies transmission dynamics

Authors: Meggan E Craft[1], Jennifer JH Reynolds[1], Ben T Hirsch[2,3], Stanley D Gehrt[4]

Affiliations: [1] University of Minnesota, Department of Veterinary Population Medicine, St. Paul, Minnesota, USA; [2] University of Florida, Department of Wildlife Ecology and Conservation, Gainesville, Florida, USA; [3] Smithsonian Tropical Research Institute (STRI), Balboa, Panama; [4] Ohio State University, School of Environment and Natural Resources, Columbus, Ohio, USA

Abstract: Infectious disease transmission often depends on the contact structure of the host population. Although it is often challenging to capture contact structure in wild animals, new technology has enabled biologists to obtain detailed temporal information on wildlife social contacts. In this study, we investigated the effects of raccoon contact patterns on rabies spread using network modeling. Raccoons can play an important role in the maintenance of rabies and the timing of raccoon rabies outbreaks in the US often exhibits a seasonal peak. We constructed a dynamic system of contact networks based on empirical data from proximity logging collars on a wild suburban raccoon population, and then simulated rabies spread across these networks. Our contact networks incorporated the number and duration of raccoon interactions. We included differences in contacts according to sex and season, and both short-term acquaintances and long-term associations. Our results demonstrate that when rabies enters a suburban raccoon population, the likelihood of a disease outbreak affecting the majority of the population is high. Both the magnitude of rabies outbreaks and the speed of rabies spread depend strongly on the time of year that rabies is introduced into the population. In contrast to previous predictions, we found that seasonal changes in social contact duration were the single most important driver of rabies seasonality. By incorporating detailed data describing the variation in raccoon contact rates into a network modeling approach, we were able to show that suburban raccoon populations are highly susceptible to rabies outbreaks, that the risk of large outbreaks varies seasonally, and that current vaccination target levels may be inadequate to prevent the spread of rabies within these populations.

Name: Amanda Feldpausch

OP65

Email: amanda.feldpausch@dph.ga.gov

Session: Epidemiology and Modeling

Country: United States

Title: Rabies response: a novel approach to human and domestic animal exposure surveillance in Georgia

Authors: Amanda Feldpausch[1], Timothy Callahan[2], Karl Soetebier[3], Julie Gabel[1], Melissa Ivey[1], Cherie Drenzek[4]

Affiliations: [1] Georgia Department of Public Health, Acute Disease Epidemiology Section, Atlanta, Georgia, USA; [2] Georgia Department of Public Health, Environmental Health Section, Atlanta, Georgia, USA; [3] Georgia Department of Public Health, Information Technology Section, Atlanta, Georgia, USA; [4] Georgia Department of Public Health, Epidemiology Section, Atlanta, Georgia, USA

Abstract: Background - Each year 250-350 animals are confirmed positive for rabies in Georgia. Timely and complete reporting of animal bites is critical in preventing human and domestic animal rabies. Although animal bites are reportable in Georgia, prior to 2012 there was no central mechanism to capture reports from numerous agencies. In 2011 the Georgia Department of Public Health (DPH) developed the Animal Bite Module (ABM), an expansion of the web-based State Electronic Notifiable Disease Surveillance System (SendSS), as a single portal for capturing all animal bite data. The ABM went live statewide in January 2013. Methods - Animal bite data pre-ABM was compared with data collected in the ABM during an equivalent period of time (14 months) to evaluate improvements in bite incident capture and documentation of follow up. A post-ABM implementation survey was administered to 471 users to assess system attributes and user satisfaction. Results - Between 1/2009 - 3/2010 (pre-ABM), 3601 electronic bite reports were entered in the non-ABM database. From 1/2014 - 3/2015, 12001 bite incidents were captured in the ABM. 10678 (89.0%) bite reports indicated all testing and human follow up for rabies exposure was complete. Of the 142 (30.0%) survey respondents, 134 (93.1%) indicated agency use of the ABM to report all animal bites. Conclusions - The number of bites reported after ABM implementation increased 3.1 times compared to a similar period pre-ABM suggesting the ABM provides a centralized user-friendly system for collecting animal bite data. Data captured in the ABM are more complete as it includes laboratory results and animal disposition information, both critical for rabies post-exposure prophylaxis risk assessment. Pre-ABM there was no mechanism for tracking completion of follow up. The ABM is a novel system which has improved animal bite surveillance in Georgia and increased opportunities for rabies prevention and control interventions.

Name: Alejandro Zaldivar Gomez

OP66

Email: al3xvet@gmail.com

Session: Epidemiology and Modeling

Country: Mexico

Title: Landscape risk factors for paralytic rabies on cattle in south of state of Mexico, Mexico

Authors: A Zaldivar-Gomez[1], A Reyes-Enriquez[2], E Muñoz-Soria[3], ME Jaimes-Benitez[4], CG Perez-Colin[4], JM Jimenez-Estrada[1], C Delgado-Urbina[1], JL Aguilar-Faisal[3]

Affiliations: [1] State Public Health Laboratory of the State of Mexico, Toluca, Mexico; [2] Faculty of Urban and Regional Planning UAEMex, Toluca, Mexico; [3] Graduate School of Medicine, National Polytechnic Institute, Mexico City, Mexico; [4] Committee of Farming Protection of the State of Mexico, Toluca, Mexico

Abstract: Bovine paralytic rabies represents severe economic losses to the cattle farming industry and a great risk to public health. The vampire bat (*Desmodus rotundus*) is one of the main reservoirs of the disease; its distribution depends on the environment and human activities. The study area comprised 14 municipalities in the south of the State of Mexico. Diverse aspects of landscape feature (socioeconomic, biophysical, animal production, dynamics of changes in land use, presence of vampire bats, rabies vaccination) were tested. The data were obtained from the Census of Housing and Population (INEGI) 2005 and 2010. Livestock production data, vaccination records, and vampire bat controls were provided by the Committee of Farming Protection of the State of Mexico. The dynamics of change in land use was developed using INEGI Land Use and Vegetation maps series III and IV (2002 and 2007 respectively). Epidemiological information was collected from ISEM Rabies Laboratory data between 1999 and 2012. The study area was developed in a grid of 76 cells of 10 x 10 km; the information obtained was applied to each cell and spatially correlated with the prevalence of positive cases. The explanatory variables were incorporated into a Multiple Linear Regression Model. Analysis of the results showed that the factors related to the presence of rabies were cells with a higher density of cattle population, these being mainly mixed production systems, change in use of grasslands, plains and the presence of intermittent rivers. These results showed the need to strengthen surveillance and control of rabies in the region.

Name: Joni Scheftel

OP67

Email: joni.scheftel@state.mn.us

Session: Epidemiology and Modeling

Country: United States

Title: One Health: rabies surveillance and epidemiology in Minnesota

Authors: Joni Scheftel[1], Stacey Schwabenlander[2], Stacy Holzbauer[1], Carrie Klumb[1], Linda Gabriel[1], Betsy Lempelius[2], Alexandra Boland[1], Gongping Liu[3], Brian Nefzger[3], Marc Schwabenlander[4]

Affiliations: [1] Minnesota Department of Health, Zoonotic Diseases Unit, Saint Paul, Minnesota, USA; [2] Minnesota Board of Animal Health, Saint Paul, Minnesota, USA; [3] Minnesota Department of Health, Public Health Laboratory, Saint Paul, Minnesota, USA; [4] Minnesota Veterinary Diagnostic Laboratory, Saint Paul, Minnesota, USA

Abstract: In Minnesota, a joint human and animal health agency rabies surveillance system has been developed to streamline response. The University of Minnesota Veterinary Diagnostic Laboratory prepares specimens for testing; the Minnesota Department of Health (MDH) Public Health Laboratory performs the testing; MDH epidemiologists handle human health issues and Minnesota Board of Animal Health veterinarians handle animal health issues. From 2003 to 2014, a total of 29,202 suspect animals were tested for rabies. Positive samples were further tested by RT-PCR and sequenced. 715 (2.5%) of the 29,202 samples tested positive, resulting in 880 exposed persons being advised to receive post-exposure prophylaxis. The highest proportion (47%, 291/618 submissions) of positive samples occurred in skunks, Minnesota's terrestrial rabies reservoir. Most skunks in the northern two thirds of Minnesota were infected with the Western Canada Skunk (WCS) variant and most skunks in Southern Minnesota were infected with the North Central United States Skunk (NCUSS) variant. Among bats, 291 (3.6%) of 8,193 submissions tested positive. Three big brown bats and a little brown myotis bat were identified with silver-haired bat variants, an eastern red bat and a northern myotis bat were identified with big brown bat variants and a silver-haired bat was identified with an eastern pipistrelle bat variant. The remainder of the bats carried the expected host variant. Over the twelve-year period, 921 raccoons were tested, and all were negative. Among domestic farm animals, cattle had the highest proportion of positive samples at 7% (50/696 submissions). Six (2.1%) of 280 horses submitted for testing were positive for rabies. Domestic carnivores (dogs, cats, and ferrets) had a lower proportion of positive samples (0.004%, 69/17,138 submissions), although they constituted the majority of submissions. Bat variants were identified in one dog, one fox and one woodchuck. All other terrestrial animals were infected with either the WCS or NCUSS variants.

Name: Jesse Blanton

OP68

Email: asi5@cdc.gov

Session: Communication and Cultural Aspects of Rabies

Country: United States

Title: Developing a transparent country risk classification system to guide pre-travel rabies vaccination recommendations

Authors: Jesse D Blanton, Emily S Jentes, Mark J Sotir, Ronnie Henry

Affiliations: Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, Atlanta, Georgia, USA;

Abstract: Developing recommendations for travel-related rabies pre-exposure prophylaxis (preEP) is a nuanced process requiring detailed risk assessments. Such risk assessments should include evaluation of a traveler's age, length of stay, planned activities, local rabies epidemiology, and availability of biologics at the destination. However, detailed information outlining the endemicity of rabies and the availability of biologics in many parts of the world can be limited. Current CDC recommendations regarding PreEP classifies destination countries into five categories: 1) canine rabies present and therefore of concern to travelers; 2) canine rabies present but not a significant concern to travelers; 3) little or no canine rabies reported; 4) rabies present in other carnivores; and 5) bat lyssaviruses present/likely/unknown. CDC subject matter experts (SMEs) in rabies and travel medicine evaluate existing literature, surveillance sources, and personal communications with regional health experts to determine individual country classifications on a regular basis. While this system provides additional information about rabies endemicity and availability of rabies biologics, it is heavily reliant on expert opinion. To improve this process and make it more data driven, an open access list of country-specific risks is needed based on standardized questions regarding endemicity of canine, wildlife, and bat lyssaviruses and the availability of biologics. This list would provide an opportunity for input from a broader pool of rabies SMEs and be more responsive to local changes in epidemiology and biologics. In addition, providing individual risk levels for primary reservoir groups (e.g. canine, wildlife, and bats) and availability of biologics will give more detailed information that can be used by travel health clinicians when counseling patients.

Name: Danielle Araujo

OP69

Email: daniellebastos@yahoo.com.br

Session: Communication and Cultural Aspects of Rabies

Country: Brazil

Title: Implementation of the project: Health and education for the control of wildlife rabies, Ceará, Brazil

Authors: Naylê Francelino Holanda Duarte[1], José Airton de Negreiros[2], Márcio Pereira de Brito[3], Wesley Cavalcante Melo[4], José Maria Oliveira[5], Bruna Holanda Duarte[6], Roberta de Paula Oliveira[1], Márcio Henrique de Oliveira Garcia[7], Benedito Neilson Rolim[8], Danielle Bastos Araujo[9], Silvana Regina Favoretto[9,10], Rossana de Aguiar Cordeiro[11]

Affiliations: [1] Center of Vector-borne Diseases, Health Department of Ceara State (NUVET-SESA), Fortaleza, Ceara, Brazil; [2] Regional Coordination of Health, Tiangua, Ceara, Brazil; [3] Regional Coordination of Education, Tiangua, Ceara, Brazil; [4] Regional Coordination of Education, Camocin, Ceara, Brazil; [5] Regional Coordination of Helath, Camocin, Ceara, Brazil; [6] Federal University of the "Semi-Árido", Mossoro, Rio Grande do Norte, Brazil; [7] Health Department of Ceara State (SESA), Fortaleza, Ceara, Brazil; [8] Central Laboratory of Public Health (LACEN), Fortaleza, Ceara, Brazil; [9] University of Sao Paulo, Laboratory of Clinical and Molecular Virology, Center for Research on Rabies, Sao Paulo, SP, Brazil; [10] Pasteur Institute of Sao Paulo, SP, Brazil; [11] Federal University of Ceara State, Fortaleza, Ceara, Brazil

Abstract: Rabies is a great challenge for Public Health in Ceara State, Northeast of Brazil. The circulation of the virus in wildlife species and the contact of these animals with humans' increases the risk of human cases, out of the five rabies cases in humans recorded from 2005 to date, four were result of aggressions by marmosets (*Callithrix jacchus*). The majority of the population is unaware of the risk that non-human primates and other wildlife species presents to rabies. Considering that wildlife vaccination is not performed in Brazil, the principal measures of control are passive surveillance, post- exposure treatment and educational activities. The aim of this project was to provide information about rabies in wildlife for children and teenagers from public schools. The project was implemented through reunions between the Secretaries of Health and Education of fourteen cities in Ceara State, where the majority of rabies cases in wildlife were registered. Through integrations between professionals of health and education the theme was discussed and the education professionals elaborated the proposals of action based in the reality of each city. As a result of the meetings and actions in the school, a series of activities was developed such as: lectures in the schools for students, teachers and employees, scavenger hunt, theater performances, information booths in the main streets of the cities, debates in classrooms, visits in houses, development of projects in science fairs, production of flyers and video presentations. Children and teenagers are a very important public for educational activities, not only because they can multiply this information in their homes but also because they can be more informed and conscious adults. The project was important to spread the knowledge about wildlife rabies to the population and can serve as a model for other important zoonotic diseases.

Name: Christine Fehlner-Gardiner

OP70

Email: Christine.Fehlner-Gardiner@inspection.gc.ca

Topic: Communication and Cultural Aspects of Rabies

Country: Canada

Title: Development of tools to aid the transition of rabies control activities from federal to provincial jurisdiction in Canada

Authors: M Kimberly Knowles[1], Kim Knight-Picketts[2], Josephine Kush[3], Maria Harding[1], Kristi Bangs[1], Lise Turbide[4], Sylvie Whitehead[4], Filomena Tittarelli[5], Denis Lafleur[5], Christine-Fehlner-Gardiner[1]

Affiliations: Canadian Food Inspection Agency: [1]Ottawa Laboratory-Fallowfield, [2]Domestic Disease Control-Policy and Programs, [3]Lethbridge Laboratory, [4]Learning Directorate-Human Resources, [5]Information Management and Information Technology

Abstract: In April 2014, responsibility for rabies disease investigation and risk assessment, sample collection and submission, and disease management in domestic animals devolved from the federal government (CFIA Operations Branch) to provincial and territorial authorities. The CFIA continued its diagnostic testing activities at the Rabies Laboratories located in Ottawa, Ontario and Lethbridge, Alberta. To facilitate this transition of rabies control activities, many informational and training tools were produced. These materials included three narrated eLearning modules on 1) disease investigation, 2) sample collection and packaging, and 3) disease management in domestic animals; a training video on proper sample packaging; and informational brochures covering general information on rabies diagnostic testing, use of the electronic submission form, sample packaging and shipping, human sample testing, and weekend/holiday testing. All materials were produced in both English and French and are freely available on DVD (by request to CFIA) and online (on the Canadian Veterinary Medical Association website). While the informational brochures are fairly specific to the CFIA Rabies Program, the eLearning modules and packaging video may be of interest to other jurisdictions in need of training tools. To permit data exchange between the CFIA's existing internal laboratory sample tracking system (LSTS) and clients external to the CFIA, an electronic solution was developed that utilizes a PDF fillable form supported by a web service and ETL strategy. This system allows test requisitions to be made electronically from a public environment, with download of sample data provided by the client directly to the restricted-access LSTS. Email notifications are automatically sent to the client following successful data submission, receipt of the sample at the laboratory, and entry of the test results into the database. This system enables secure, accurate and efficient information exchange between the laboratory and its clients.

Name: Marco Vigilato

OP71

Email: vigilato@paho.org

Session: Communication and Cultural Aspects of Rabies

Country: Peru

Title: Technological tools for strengthening human rabies prophylaxis in the Americas Region

Authors: O Chavez-Inagaki[1], SE Recuenco Cabrera[2], MAN Vigilato[3]

Affiliations: 1 Peruvian University Cayetano Heredia (UPCH), Lima, Peru; [2] National Institute of Health, Lima, Peru; [3] Pan American Health Organization, Lima, Peru

Abstract: Rabies is a viral, acute, progressive and deadly encephalitis caused by a lyssavirus, but 100% preventable by applying appropriate pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) using rabies immunobiologicals, because despite technological advances, still the lack of successful treatment when the patient manifest symptoms. Worldwide, about 59,000 deaths occur annually from human rabies, causing great social-economic impact. Therefore, it is important that health professionals are properly trained and actualized to promote rabies control and prevention to achieve its elimination. Considering the mentioned the Pan American Health Organization (PAHO) has developed an online course with 3 modules to guide and strengthen knowledge, and update appropriate technical competences for the correct use for PEP and PrEP against rabies. This didactic initiative provides to the online participants, with no cost, full access to educational tools such as videos by trained professionals with experience on progress in the region of the Americas, different guidelines from all over the region and scientific material consultation. At the end of each module, there are evaluations required for reinforce the knowledge acquired. A certificate of participation is granted when finalized the virtual course. In addition, PAHO has developed an "application" for Smartphones, based on the Expert Consultation on Rabies WHO (2013), in order to guide the health professional responsible for attention of persons exposed to rabies risk in Americas. It is possible to consult the proper management of a exposure case to rabies virus, and find updated guidelines for use appropriate PEP. These tools can contribute to achieve the elimination of human rabies in the Region of the Americas and promoting public health interventions, providing a rapid and timely attention to the population at risk of rabies.

Name: Hunter Bodenchuk

OP72

Email: michael.j.bodenchuk@aphis.usda.gov

Session: Communication and Cultural Aspects of Rabies

Country: United States

Title: Vampire bat community communication and outreach video

Authors: HA Bodenchuk[1], RS Garces[2], RB Chipman[3], L Lecuona[2], MJ Bodenchuk[4]

Affiliations: [1] Texas A&M AgriLife Extension-Wildlife Services, San Antonio, Texas, USA; [2] US Department of Agriculture, International Services, Mexico City, Mexico; [3] US Department of Agriculture, Wildlife Services, National Rabies Management Program, Concord, New Hampshire, USA; [4] US Department of Agriculture, Wildlife Services, San Antonio, Texas, USA

Abstract: Bats have had on important place of ancient cultures and today the vampire bat continues to contribute to popular culture due to its ability to feed on mammalian blood. Vampire bats pose a significant rabies threat to humans and domestic animals throughout their range. Recent evidence of northward range expansion in parts of Mexico has been documented and climate and population models now predict vampire bats could reach the US/Mexico border in the next decade. Communication and outreach about vampire bat and rabies ecology rabies is a critical component for managing potential future impacts to public and animal health. With the potential expansion of vampire bat populations into the U.S., an opportunity to educate landowners, veterinarians and the wildlife community exists. A short 5 minute educational DVD targeting ranchers, landowners and homeowners along the Mexico–U.S border about vampire bats and rabies has been produced by USDA, APHIS, Wildlife Services (WS) program in Texas; APHIS, International Services and the WS National Rabies Management Program. Both an English version and Spanish version will be made available to the public. In this presentation we outline the details of the production including editing and production costs as well as strategies for distribution and measuring the impact of our outreach efforts. The presentation will also include the first public viewing of the DVD.

Name: Alan Jackson

OP77

Email: ajackson2@hsc.mb.ca

Session: Pathogenesis and Molecular Epidemiology

Country: Canada

Title: Critical appraisal of the Milwaukee protocol for rabies: this failed approach should be abandoned

Authors: Frederick A Zeiler and Alan C Jackson

Affiliations: University of Manitoba Health Sciences Centre, Winnipeg, Manitoba, Canada

Abstract: On the basis of only one case report, the Milwaukee protocol has been attributed to survival in rabies encephalitis and promoted relentlessly despite a lack of scientific evidence supporting its therapeutic measures. The protocol has changed significantly since its inception. Over the 10 year period from 2005 to 2014 there have been at least 29 documented failures and no subsequent survivors other than in situations where survival occurred without use of the protocol (e.g. rabies vaccine given prior to the onset of disease). In addition, fatal cases related to illness due to rabies have been incorrectly claimed to be survivors. We have reviewed the literature with reference to specific treatment recommendations made within the protocol. Therapies suggested in the Milwaukee protocol include amantadine, ketamine infusion, therapeutic coma, and the screening/prophylaxis/management of cerebral vasospasm. The current literature fails to support an important role for excitotoxicity or cerebral vasospasm in rabies encephalitis. None of these therapies can be substantiated in rabies or in other forms of acute viral encephalitis. Serious concerns over the recommendations of the current protocol are warranted in light of weak scientific rationale and a documented lack of efficacy over the last decade. All of the recommendations made in the Milwaukee protocol warrant serious reconsideration before any future use of this failed protocol. Continued use of this protocol has actually impeded progress in the development of effective therapies for human rabies. An improved understanding of rabies pathogenesis may prove helpful in the development of novel effective therapies for rabies.

Name: Françoise Guinet-Morlot

OP80

Email: francoise.guinet@sanofipasteur.com

Session: Bats and Lyssavirus Host and Virus Diversity

Country: France

Title: A glycoprotein G ELISA as an alternative to the NIH potency test of human rabies vaccines

Authors: F Guinet-Morlot, N Moreno, C Manin, M Chabaud-Riou, P Riou

Affiliations: Sanofi Pasteur, Campus Merieux, Marcy l'Etoile, France

Abstract: As part of the 3Rs initiative, the European Partnership for Animal Alternatives (EPAA) has initiated a project for the replacement of the human rabies vaccines in vivo potency test – the National Institutes of Health (NIH) test – by an in vitro test. Under this EPAA initiative, an International Working Group of 25 international experts from government, industry, and academia was created in October 2012 to coordinate preliminary studies aimed at selecting the appropriate in vitro test to be used in an international collaborative study for the replacement of the NIH test. The major correlate of protection of rabies vaccines is the serum titer of neutralizing antibodies against the unique transmembrane glycoprotein G. The ability of the rabies virus glycoprotein G to induce both virus neutralizing antibodies and protection depends on the preservation of its three-dimensional structure. Several ELISAs quantifying glycoprotein G have been identified by the International Working group as appropriate in vitro tests for the replacement of the NIH test. Among those, the ELISAs developed by manufacturers aim at defining the formulation target and testing the actual content of glycoprotein G per human vaccine dose. During the development of its next-generation rabies vaccine VerorabVax™, Sanofi Pasteur has set up an ELISA able to quantify Glycoprotein G and detect structural alteration resulting from heat inactivation, beta-propiolactone treatment or chemicals. At this stage, this test is considered an appropriate candidate for the replacement of the NIH Test. The corresponding data will be presented.

Name: Samira Maria Achkar

PP01

Email: samira_achkar@yahoo.com.br

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: Brazil

Title: Evaluation of histopathological changes and the presence of inclusions in different fragments of central nervous system of horses naturally infected by rabies virus

Authors: SM Achkar[1], ER Fernandes[1], F Guedes[1], E Mori[1], MIS Duarte[2]

Affiliations: [1] Pasteur Institute of São Paulo, Brazil; [2] University of São Paulo, Faculty of Medicine, Brazil

Abstract: The more difficult of rabies diagnosis are described in the equine species, often on the basis of the fragment analyzed. The present study aimed to evaluate histopathologic changes and the presence of inclusions in different fragments of equine brain tissue naturally infected by rabies virus, previously diagnosed positive through the classical techniques (direct immunofluorescence and viral isolation in mice). Ten samples from Central Nervous System were selected, of which included fragments of hippocampus, cerebral cortex, cerebellum and brain stem. The histopathological evaluation and the presence of inclusions were performed by employed hematoxylin and eosin staining. The presence of inclusions was observed in at least one fragment of each sample, being more frequent in the cerebellum, followed by the brainstem. The neuronal degeneration was observed in all fragments evaluated, however, the processes of neuronophagia and microglial nodules were more frequent in the brainstem, and in lesser intensity in the cerebellum. The inflammatory changes recorded were: meningitis; vasculitis; perivasculitis and gliosis, being the most affected fragment the brainstem. The degree of inflammation prevalent was discreet, however, in the brainstem was observed a moderate intensity. The presence of inclusions was observed in neurons located in areas with a few inflammatory reactions, which suggests that the histopathological changes are not resulting only by the presence of the virus, but due to the inflammatory process seen in the disease. However, due to the rapid progression in the clinical course of rabies in horses, the degree of histopathological changes observed in this species is mostly discreet, in this way, it is necessary the use of classical techniques for confirmation of the disease, because the absence of these changes does not discard the infection.

Name: Fernanda Guedes

PP02

Email: fernandaguedes@usp.br

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: Brazil

Title: Detection of rabies virus antigen by rapid immunohistochemistry test: experience of the Pasteur Institute, Brazil

Authors: F Guedes[1], NHF Centoamore[2], ER Fernandes[1], SM Achkar[1]

Affiliations: [1] Pasteur Institute of São Paulo, Brazil; [2] University of São Paulo, Faculty of Veterinary Medicine and Zootechny, Brazil

Abstract: The development of diagnostic techniques for rabies more suitable, the application in laboratory routine in developing countries, has undergone major advances. The objective of the present study was to perform the rapid immunohistochemistry test (rIHQt) for rabies diagnostic. The sample set used in this study consisted of 37 central nervous system (CNS) samples obtained from the Laboratory of Virology, Pasteur Institute, Brazil and derived from the following mammalian species: cat (1), cattle (31), horse (2), bats (*Artibeus lituratus*; n=2 and *Cynomops planirostris*; n=1). The animal specimes performed at the Virology and Immunohistochemistry Laboratories, Pasteur Institute, was approved by the Animal Ethics Committee (Pasteur Institute, São Paulo, Brazil) for the use of animals for research in diagnostic procedures under number 04/2014. Polyclonal mouse anti-rabies virus primary antibody (Evandro Chagas Institute, Pará, Brazil) followed by incubations with biotinylated link antibody and peroxidase-conjugated streptavidin (LSAB2 kit). Negative controls were included parallel slides from CNS processed as described above, omitting the primary antibody. All the samples included in this study were also processed for the direct fluorescent antibody (DFA) test, considered the gold standard assay for rabies diagnosis. The results of the rIHQt were produced while the DFA result was not known to the operator at the time when the immunohistochemistry results were interpreted. All samples evaluated presented positive immunolabeling for rabies virus by rIHQt. Of the 37 samples tested in the study, the DFA test produced two false-negative results in comparison to the rIHQt. The first sample was collected from a cat and the second from a horse in the State of São Paulo, Brazil. These two samples were confirmed positive for rabies by genetic sequencing. Our partial results suggest that this protocol for rapid immunohistochemistry test could be applied to rabies diagnostic, however, are necessary more studies to evaluate its specificity and sensitivity.

Name: Keila Iamamoto

PP03

Email: keilavt@yahoo.com.br

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: Brazil

Title: Evaluation of sample concentrations in the accuracy of the rabies virus isolation in N2A cell culture

Authors: Tatiane de Cassia Pardo de Souza, Adriana Candido Rodrigues, Willian de Oliveira Fahl, Karen Miyuki Asano, Karin Corrêa Scheffer, Samira Maria Achkar, Enio Mori, Keila Iamamoto

Affiliations: Pasteur Institute of São Paulo, São Paulo, Brazil

Abstract: Besides the replacement of animal use, the viral isolation in cell culture (VICC) has lower cost and more agility in obtaining results. However, factors related to the quality and preparation of samples have direct influence in the technique accuracy. Thus, this study evaluated the application of different concentrations of bovine CNS suspensions in N2A cell cultures, in order to improve the sensitivity of the technique. Twenty samples were selected by systematic sampling method among positive samples of bovines by FAT in 2013. CNS fragments suspensions were prepared at concentrations of 5, 10, 20, 30, 40 and 50% (w/v), which were inoculated in two different moments to evaluate reproducibility in VICC. Loss of cell confluence and the viral titer of each sample were also evaluated. In the VICC all samples were positive in at least one of the concentrations. At 5, 10 and 20% concentrations, the integrity of cell monolayer was observed for all samples. However, a loss of cell confluence was observed from the concentration 30% on, while at the concentration 50% a complete cell confluence loss was observed. Regarding the reproducibility, from 5 to 20% concentrations an agreement over 90% was observed, while from 30% concentration on, the agreement decreases achieving 50%. These results demonstrated that using the concentrations at 5 to 20%, a higher sensitivity was observed when compared to the concentrations at 30 to 50%. This study reinforces the importance of the sample preparation, which can compromise the VICC technique accuracy due to cell fragility.

Name: Iana Suly Santos Katz

PP04

Email: ianasuly@gmail.com

Session: Lyssavirus Diagnostics and Emerging Technologies

Country: Brazil

Title: New potential diagnostic tool for rabies

Authors: Iana Suly Santos Katz[1], Luciana Botelho Chaves[1], Orlando Garcia Ribeiro[2], Karin Corrêa Scheffer[1], Leo Iwai[2]

Affiliations: [1] Pasteur Institute of São Paulo, São Paulo, Brazil; [2] Butantan Institute, Immunogenetics Laboratory, São Paulo, Brazil

Abstract: Rabies is a major zoonotic viral disease, and rapid and effective diagnoses are particularly important. Currently the techniques recommended by WHO in rabies laboratory diagnosis have several limitations, mainly ante-mortem rabies diagnosis. Other methods have been used in research laboratories improving the performance of biological assays. In this context, modern advances in molecular technology promise to revolutionize the diagnosis of rabies. In this study, we have combined mass spectrometry, the fast data acquisition, intelligent interpretation software and protein databases for diagnosis of rabies. The database search identified different peptides from nucleoprotein, phosphoprotein, RNA-dependent RNA polymerase, glycoprotein, and matrix protein of wild rabies virus isolates from different reservoirs. Here we showed that proteomic approach could be a good alternative in both rabies research and diagnosis, as also for discovery of protein biomarkers opening the way for the development of novel immunodiagnostic tests and vaccine.

Name: Zaheer Hussain

PP06

Email: zaheersbs@gmail.com

Session: Human Rabies and Prophylaxis

Country: Pakistan

Title: Factors which sharpens the killer bites of rabies in Pakistan

Authors: Zaheer Hussain[1], Muhammad Saleem Haider[1], Zafar Ul Ehasan Qureshi[2]

Affiliations: [1] Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan; [2] Veterinary Research Institute, Lahore, Pakistan

Abstract: Rabies is a vampire which is quenching his thirst by human lives since pre historic times. Although the reason of great success of rabies virus is its ability to switch multiple hosts (theoretically rabies virus can infect all warm blooded animals) but real factors which help this deadly virus to terminate around sixty thousand lives every year around the world is care free attitude of people in developing world and ignorance of some public health professionals about rabies vaccines and how to manage a case of dog bite. The survey conducted from public health professional (medical practitioners' and paramedical staff) showed that majority of them don't know about first line management in case of rabid dog bite, antirabies vaccines and antirabies immunoglobulins in urban as well as rural areas. The common people are almost completely ignorant about preventive measures against rabies. Recently we attend rabies suspected case in a public hospital. The person was bitten by a dog and went for vaccination against rabies. The local medical practitioner injected antirabies vaccine (Verorab) intraperitoneally. Neither the patient nor the practitioner tried or emphasis for further dose. Six weeks after vaccination, the patient developed rabies and brought to the hospital where he died within four days. The saliva sample of the patient was collected and rabies virus was confirmed through RT-PCR and subsequent sequencing. The phylogenetic analysis revealed that virus was similar to other rabies virus isolates circulating in Pakistan. This death could be avoided if quack did not inject intramuscular vaccine intraperitoneally or simply guide patient about right place for vaccination. The patient follows the complete schedule of rabies vaccination from a reputed place. How many dies every day like this, nobody knows.

Name: Ha-Hyun Kim

PP08

Email: hahyun@korea.kr

Session: Human Rabies and Prophylaxis

Country: South Korea

Title: A combination treatment with mouse monoclonal antibody and adenovirus bicistronically expressing porcine interferon-α and interferon-γ enhanced protection against lethal peripheral rabies challenge in mice

Authors: Ha-Hyun Kim, Dong-Kun Yang, Hyun-Ye Jo, In-Soo Cho

Affiliations: Animal and Plant Quarantine Agency, South Korea

Abstract: Rabies is highly fatal once symptoms are expressed in mammals. Successful cure of rabies is depending on appropriate treatment to an exposure. In order to investigate the potential of mouse monoclonal antibody having neutralizing capacity and adenovirus bicistronically expressing porcine interferon-α and interferon-γ (Ad-porcine IFN-αγ) as antiviral agents against rabies virus (RABV), mice were challenged with lethal RABV by the intramuscular route in femoral muscle and then were administrated with 0.1 mL of monoclonal antibody (neutralizing titer 20 IU/mL) and 0.1 mL of Ad-porcine IFN-αγ (107.5 TCID50/mL) after the challenge 6 h. Groups receiving both monoclonal antibody and Ad-porcine IFN-αγ (33.3%) had higher survival rates than the groups receiving only monoclonal antibody (20%). When BHK-21 cells were treated with Ad-porcine IFN-αγ (106 TCID50/mL) at 16 h before RABV inoculation (CVS11, 100 TCID 50/mL), the RABV fluorescent foci were remarkably reduced. These results indicate that anti-viral effects of combination treatment with mouse monoclonal antibody and Ad-porcine IFN-αγ against RABV were enhanced in 4-week-old BALB/c mice compared to that of single treatment of monoclonal antibody.

Name: Heidi Smith

PP09

Email: heidi.smith@umassmed.edu

Session: Human Rabies and Prophylaxis

Country: United States

Title: Neutralization of North American rabies isolates by R172, a human monoclonal antibody cocktail

Authors: Heidi L Smith[1], Todd G Smith[2], Susan M Moore[3], Lauren Greenberg[2], William C Carson[2], James A Ellison[2], Adam Buzby[1], Mark Klempner[1], Yang Wang[1], William D Thomas Jr[1], Deborah C Molrine[1]

Affiliations: [1] MassBiologics of the University of Massachusetts Medical School, Boston, Massachusetts, USA; [2] Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, Atlanta, Georgia, USA; [3] Kansas State University Rabies Laboratory, Manhattan, Kansas, USA

Abstract: The development of novel anti-rabies biologics requires demonstration of broad neutralizing activity considering the diversity of variants circulating throughout terrestrial and arboreal reservoirs in North America. Two human monoclonal antibodies (RAB1, RAB2) that recognize distinct glycoprotein epitopes were evaluated singly and in combination as a 1:1 cocktail (R172) against 39 rabies isolates by RFFIT. Neutralizing activity was quantified as the concentration at which 50% neutralization was observed (EC50, µg/mL). Among 15 carnivore isolates evaluated, RAB1 demonstrated EC50 values <1µg/mL for 12 (80%); the remaining 3 (20%) were neutralized at EC50s >1µg/mL. RAB2 neutralized 14 terrestrial isolates (93%) at EC50s <1µg/mL, and R172 neutralized 100% of terrestrial isolates at EC50s <1µg/mL. Of 24 bat derived isolates evaluated, RAB1 demonstrated EC50s <1µg/mL for 6 (25%), 17 (71%) had EC50s >1µg/mL and a single isolate (4%) was not neutralized at the highest concentration tested; this isolate had a laboratory-acquired RAB1 epitope mutation. For RAB2, 11 bat isolates (46%) were neutralized at EC50s <1µg/mL, 9 (38%) at >1µg/mL, and 4 (17%) bat isolates (each isolated from a Lasiurus sp. bat) were not neutralized at the highest RAB2 concentration tested. R172 neutralized all 24 bat isolates and had EC50s <1µg/mL for 12 (50%); 11 of the 12 with R172 EC50s >1µg/mL were from Lasiurus bat species. A higher concentration of HRIG was also required to neutralize Lasiurus isolates (median EC50 0.134 IU/mL, range 0.012-0.259) compared to other bat (median 0.029 IU/mL, range 0.003-0.190) and carnivore isolates (median 0.014 IU/mL, range 0.003-0.100). These in vitro data indicate that R172 demonstrates a breadth of neutralizing activity comparable to HRIG. In vivo neutralizing activity of R172 against carnivore and bat rabies isolates, including isolates from Lasiurus sp., will be evaluated in a lethal hamster challenge model of post-exposure prophylaxis.

Name: Miguel Iguala-Vidales

PP11

Email: miiguala@yahoo.com.mx

Session: Human Rabies and Prophylaxis

Country: Mexico

Title: Human case of rabies in the State of Chihuahua from a skunk bite

Authors: VM Iguala[1], MM Melo[1], SD Martínez[1], BA Sandoval[1], MI Padilla[1], SM Gómez[1], TR Terán[1], RB Escamilla[1], VI Animas[1], LS Chávez[1], PF Vargas[2], CV Gutiérrez[2], FA Chávez[2], LB Torres[1], PM Vázquez[1], CN Arechiga[1], MI López[1], QA Díaz[1]

Affiliations: [1] Institute of Epidemiological Diagnosis and Reference, SALUD/InDRE, México D.F.; [2] National Center for Preventive Programs and Disease Control, SALUD/CENAPRECE, México D.F.

Abstract: Male patient, 45 years old, originally from the indigenous town of Ariachi, municipality of Batopilas, Chihuahua. On April 9th of this year, the patient was sent to the Polanco Rural Medical Unit, IMSS PROSPERA, diagnosed with a bite from a wild animal (skunk, confirmed). He began his condition two months ago. With a bite from a wild animal (skunk), in the right maxillary region, the wound has a length of 3 centimeters, serious risk of exposure, we interviewed 2 teenagers that are sons of the patient, but they are not fluent in Spanish, then they got help of the indigenous nursing staff. They both comment that 6 or 7 days ago, he began with the signs and symptoms of headache, unquantified fever, pain at the site of the bite, excessive sweating, fatigue, abundant salivation, psychomotor agitation, aerophobia, hydrophobia, paresis, dehydration, low tolerance to oral via and limitation for ambulating. Also were reported the presence of convulsive crisis of the tonic-clonic type. Patient denied history of important chronic degenerative conditions, no allergies, no transfusion, no fractures, "O" RH + blood type. On arrival at the health unit he is in very poor condition, restless, Glasgow of 15, mydriatic pupils, with anisocoria, 39.5 C temperature, presence of tachycardia, bradycardia 30X, 45% oxygen saturation, hypotension, trismus, and seizure. Patient managed with hydration, antibiotics, and application of 2 intramuscular vero cell dosages. The patient meets the standard for operational definitions as a suspect and probable case of rabies. The samples processed were saliva, scalp biopsy, cerebrospinal liquid, corneal impressions and brain, resulting in isolation and confirmation of rabies by IFD. Genetic characterization and phylogenetic reconstruction of the isolated virus was performed, confirming skunk variant of the state of Chihuahua.

Name: Marco Vigilato

PP12

Email: vigilato@paho.org

Session: Human Rabies and Prophylaxis

Country: Peru

Title: Prevention of sylvatic human rabies in the Americas

Authors: Ana Maria Navarro[1], Sergio Recuenco[2], Ricardo Lopez[2], Paulo Pachas[2], Oswaldo Cabanillas[1], Daphne León[3], Marco Vigilato[4]

Affiliations: [1] Zoonosis Strategy Department, Ministry of Health of Peru, Lima, Peru; [2] National Health Institute, Ministry of Health of Peru, Lima, Peru; [3] Peruvian University Cayetano Heredia, Lima, Peru; [4] Veterinary Public Health Area, PANAFTOSA, Pan American Health Organization, Lima, Peru

Abstract: Objective: Consolidate relevant information to the implementation of pre-exposure prophylaxis (PrEP) to rabies transmitted by hematophagous bat in vulnerable populations in the Americas. Materials and methods: was conducted a deep literature review on the impact and the situation of human rabies transmitted by hematophagous bat in the Americas and the use of PrEP as an alternative disease prevention. Results: The rage of wild origin remains a serious public health problem in Latin America associated cases the hematophagous bat have killed at least, 170 people in the last 10 years (official numbers). Amazonian indigenous populations are considered high risk for the disease because of its social, economic and cultural characteristics. PrEP is recommended for people living in endemic areas through individual or en massive application. In Latin America, they have experienced PrEP locally in Ecuador, Colombia and Peru. In 2011, Peru implemented the Plan of Rabies Vaccination of pre-exposure rabies risk communities in the Amazon region, the Plan considered the application of cell-culture vaccines to 15.242 people in the provinces of Condorcanqui and Bagua. 13.986 people completed the 3 doses of vaccine and 99.6% achieved protective antibody levels. From this experience 3 more regions of Peru joined the Plan, becoming the most experience in implementing PrEP to prevent rabies, with a total of 90.877 persons immunized in the Peruvian Amazonian region. Conclusions: The implementation of large-scale PrEP risk areas is an effective tool for reducing cases of human rabies from the wild. It is necessary to define criteria for identifying risk areas that should be prioritized.

Name: Ana Martins

PP13

Email: caroltardin@gmail.com

Session: Human Rabies and Prophylaxis

Country: Brazil

Title: Human anti-rabies care in the Federal District, a quantitative assessment of the notifications after the systematization in SINAN

Authors: Ana Martins, Ana Grisoto, Teresa Segatto, Suzana Ilha, Marilia Carvalho, Ros Mossri, Suênia Sampaio

Affiliations: Health State Secretary of the Federal District, Brazil

Abstract: Rabies is an obligatory reportable disease in Brazil, as usual as the human anti-rabies attendance. Currently, notification of human anti-rabies attendance is performed by inserting the data in the Notifiable Diseases Information System (SINAN), which gathers information on notifiable diseases in the country. Until 2014, in the Distrito Federal (DF), that notification was made through bookmarks manually filled at the site of care, with monthly payment of statistics to the District surveillance sector. The notification of human anti-rabies care was implemented in SINAN in 2010, and started in the DF in January 2015. The objective of this study was to quantitatively evaluate the systematization of human anti-rabies care in the Federal District. This is a cross-sectional, retrospective, epidemiological and descriptive study. The data were recorded from 2006 to 2014 and compared to the period between January and June 2015. The information was allocated in Microsoft Office Excel®, version 2007, and was analyzed by a control diagram. The variables were total number of human anti-rabies attendance, treatment's dropout number and the number of biting animals. It was observed that the number of visits reported in SINAN in 2015 fell considerably, below the lower limit calculated from the average of previous years. So did the number of abandonments of treatment and number of biting animals. However, the number of rabies vaccine doses remained in the expected range. The SINAN is a good tool to monitor the grievance. There was a reduction in the number of visits, since notification errors as duplicity have been fixed. As the number of vaccine doses remains in the expectations, it can be explained by the fact that this data was the only in the manual statistics that was already extracted from a computerized system (API-WEB).

Name: Camila Appolinario

PP14

Email: jane@fmvz.unesp.br

Session: Pathogenesis and Molecular Epidemiology

Country: Brazil

Title: Rabies virus in salivary gland and organs from naturally infected ruminants

Authors: CM Appolinario, FFC Castro, MG Peres, MR Mioni, AF Vicente, VC Pelicia, CR Fonseca, BD Ribeiro, J Megid

Affiliations: São Paulo State University, Faculty of Veterinary Medicine and Animal Science ("Julio de Mesquita Filho"), Botucatu, SP, Brazil

Abstract: In 2014 until June 2015, cases of rabies in sheep and cattle, due to hematophagous bat (*Desmodus rotundus*) were being reported in the central-west region of São Paulo state in Brazil. Some animals had evident cicatricial lesions done by the hematophagous bat but most of them didn't present those marks. Sheep (n=4) and cattle (n=5) of different ages from different locations were brought to the Infectious Disease Service -Veterinary Hospital of the Faculdade de Medicina Veterinaria e Zootecnia- UNESP in Botucatu. The animals presented neurological that evolved to death in variable period of time. In two of the bovines saliva was collected during the evolution period. After death, brain, spinal cord, CSF, liver, kidneys, spleen and lungs were collected to rabies virus detection from all of the affected animals. The samples were extracted with a commercial kit (RTP® DNA/ RNA Virus Mini Kit - Invitrogen®) and submitted to real-time RT PCR using specific primers to detect rabies virus nucleoprotein gene. All the nine brain samples were positives with CT ranging between 10 to 26 cycles. Spinal cord positivity occurred in all sheep and in one bovine; CSF of one sheep and 2 bovines were collected and only one, belonging to the bovine, resulted as positive. One out in 2 bovines had oral swabs positive. Salivary glands were positive in 2 out 5 bovines with relative low concentration of virus (CT ranging between 22-30 cycles). Samples of kidney, liver, lung and spleen resulted as negative in all animals. Considering these results it's clear that rabies virus has the SNC as the elected site for replication but the detection of the virus in the salivary glands as well as in the oral swabs of rabid bovines reinforce the risk of transmission by this species.

Name: Iana Suly Santos Katz

PP15

Email: ianasuly@gmail.com

Session: Pathogenesis and Molecular Epidemiology

Country: Brazil

Title: Pathogenicity of rabies virus isolated from the main rabies reservoirs in Brazil

Authors: Natalia Langenfeld Fuoco[1], Karen Miyuki Asano[1], Luciana Botelho Chaves[1], Orlando Garcia Ribeiro[2], Karin Corrêa Scheffer[1], Iana Suly Santos Katz[1]

Affiliations: [1] Pasteur Institute of São Paulo, São Paulo, Brazil; [2] Butantan Institute, Immunogenetics Laboratory, São Paulo, Brazil

Abstract: Rabies is an acute progressive, which usually causes a fatal encephalomyelitis in many species of mammals. Rabies virus (RABV) is a member of the Lyssavirus genus within the Rhabdoviridae family. Based on the clinical presentation, rabies can be classified into encephalitic (furious) or paralytic (numb) rabies. According to some studies, the diversity of clinical manifestations of rabies may result from different neuropathogenic mechanisms of the virus. In this context, this study aims to determine the differences in RABV pathogenicity found in strains of viruses isolated from different reservoirs species of Brazil. For this purpose, the RABV variant associated with *Desmodus rotundus* (antigenic variant 3-AgV3), *Eptesicus* sp, *Canis familiaris* (antigenic variant 2- AgV2), *Callithorix jacchus* or fixed virus (CVS-31) were inoculated by intradermical route in different group mice with, and typical clinical signs of rabies were observed daily for 40 days. Our results suggest that following RABV inoculation of the antigenic variants isolated from marmoset and *Eptesicus* bats showed to be less pathogenic than the other variants studied, exhibited a long incubation period and the lowest mortality rates. Outbreak of aggression was observed only in a small percentage of animals inoculated with AgV isolated from marmoset and AgV3, as well as ruffled fur was the most prevalent in animals inoculated with CVS. There was no significant difference among the groups for other clinical symptoms (disordered movement, trembling and complete paralysis). These results can contribute to a better understanding of rabies pathogenesis in different hosts.

Name: Jane Megid

PP16

Email: jane@fmvz.unesp.br

Session: Pathogenesis and Molecular Epidemiology

Country: Brazil

Title: Brain gene expression profile of variant 2 street rabies virus in infected mice

Authors: CM Appolinario[1], FA Marchi[2], MG Peres[1], BD Ribeiro[1], MR Mioni[1], AF Vicente[1], CR Fonseca[1], SR Rogatto[2], J Megid[1]

Affiliations: [1] São Paulo State University, Faculty of Veterinary Medicine and Animal Science ("Julio de Mesquita Filho"), Botucatu, SP, Brazil; [2] NeoGene, AC Camargo Cancer Center, São Paulo, SP, Brazil

Abstract: Rabies pathogenesis has been studied in many different aspects but a precise understanding of the mechanisms involved in this disease was not reached. Rabies virus strains present different levels of neurovirulence and thus differ in the levels of inflammation, apoptosis and neural degeneration especially when street rabies virus is compared to a fixed strain. The aim of this study is to evaluate in two different moments the gene expression profile in brains of mice infected with a variant 2 street rabies virus. A total of 20 C57/BL6 mice, SPF, female, 4-6 weeks-old, were inoculated in gastrocnemius muscle with 100uL of rabies virus inoculum (LD50 10-6,66); at the same time 10 mice were inoculated with saline (controls). Inoculated animals were separated in two groups, one with 10 animals observed for 30 days for lethality. In the other group 5 animals were sacrificed at 5 and 10 days post-inoculation (d.p.i.) and whole brain collected for microarray analyse. Extraction of total RNA was performed with a commercial kit; Gene expression analysis was performed using the microarray GeneChip® Mouse Gene 2.0 ST Array from Affymetrix®. Canonical pathways, networks and gene functions were evaluated using Ingenuity Pathways Analysis (IPA). For data analysis, the parametric statistical t-test with $p < 0.05$ and 1000 permutations were applied. At 5 d.p.i the analysis revealed 44 genes up-regulated and 23 down-regulated; 7 main canonical pathways were identified including T cell apoptosis, B cell modulation, cytokine modulation, humoral/celular response, neurotransmitters modulation and neuronal activity. At 10 d.p.i 231 genes were up-regulated and 213 were down-regulated; 13 main canonical pathways were identified being related to interferon signaling, dendritic cell maturation, communication between innate and adaptative immune response, toll-like receptor signaling, activation of IRF, cellular apoptosis, T cell activation and T cell response. Despite the activation of several cell cycles involved in host mechanisms of defense the lethality observed was 100% demonstrating the failure in rabies virus infection control.

Name: Scott Bender

PP17

Email: scottbender@navajo-nsn.gov

Session: Immunology and Vaccines

Country: United States

Title: Rabies vaccination of puppies - how young is too young…?

Authors: SC Bender[1], DL Bergman[2], CE Rupprecht[3]

Affiliations: [1] The Navajo Nation, Veterinary Program, Chinle, Arizona, USA; [2] US Department of Agriculture, Wildlife Services, Arizona, USA; [3] The Wistar Institute, Philadelphia, Pennsylvania, USA

Abstract: Canine rabies vaccination is one of the most effective strategies to prevent human rabies. However, current recommendations suggest vaccination of dogs no earlier than 3 or 4 months of age, depending upon the local jurisdiction, based in part upon guidelines of the U.S. Compendium of Animal Rabies Prevention and Control. In this study, 27 dogs, between 8-10 weeks of age, were recruited from volunteer clients to evaluate the potential utility of early vaccination against rabies, at their first visit for a 'well puppy' vaccination program at a Navajo Nation Veterinary Clinic. During the initial visit, the puppies had a serum sample collected and were vaccinated parenterally against rabies using Nobivac®-3, an inactivated, three-year Rabies vaccine, manufactured by Pfizer Animal Health. Puppies returned to the clinic for booster vaccinations of parvovirus and distemper virus over 4 months, then at 4 months of age, a follow-up serum sample was collected and an official rabies vaccination was administered for licensing. All 27 puppies that started the study, demonstrated an antibody titer less than 0.05 IU/ml at intake. At the conclusion of the study, 20 of the 27 project puppies completed the "well puppy" program and were tested. Of these, 18 of the 20 puppies had evidence of rabies virus neutralizing antibodies, ranging from 0.14 to 13.0 IU/ml. Such preliminary data support the concept that puppies are immune-competent earlier than 3 months, and should be vaccinated regardless of age. Young dogs make up a critical cohort of canine populations, particularly in developing countries and may be missed during mass vaccination programs if recommendations require them to be three to six months old. Considering the safety and potency of modern veterinary vaccines, all animals, regardless of age should be covered as part of annual immunization campaigns to prevent, control and eliminate canine rabies.

Name: Rolan Davis

PP18

Email: rdavis@vet.k-state.edu

Session: Immunology and Vaccines

Country: United States

Title: RVNA titer of 0.5 IU/mL in dogs and cats should be allowed in lieu of routine vaccination

Authors: Rolan Davis[1], Mike Moore[1], Susan Moore[1], Qing Kang[2], Christopher Vahl[2], Tom Bunn[3]

Affiliations: [1] Kansas State University Rabies Laboratory, Manhattan, Kansas, USA; [2] Kansas State University, Department of Statistics, Manhattan, Kansas, USA; [3] Kansas State University (Retired)

Abstract: Recent work by Moore, et al shows that the immune response to booster vaccination is similar in dogs and cats with current vaccinations or expired vaccinations, leading to changes in the way pets exposed to rabies are handled. At the time this work was presented to the Compendium of Animal Rabies Prevention and Control (Compendium) committee, members asked whether this would lead to a 'vaccinate once and forget' attitude among pet owners. Vaccination of pets keeps the incidence of this disease in dogs and cats to a minimum and is still the most important step to avoid human exposure to this virus in the United States. For all the benefits afforded by vaccination against rabies, there are recognized risks to multiple vaccinations, including anaphylaxis, immune mediated hemolytic anemia and inject site sarcomas in cats. As society evolves and pets become more important in peoples' lives, these side-effects are becoming less tolerated. Currently, the AVMA, AAHA and AAFP all have adopted standards for allowing for the use of serologic assays to determine the necessity of booster vaccination for all core vaccinations, except rabies. The reasons cited by these organizations for the lack of serologic antibody titer standards for revaccination against rabies are state and local laws mandating routine (every 1 or 3 years) vaccination. Most municipalities in the United States require licensing of dogs, and to a lesser extent cats, which is dependent upon a current vaccination certificate. A careful review of existing data shows that antibody titers against the rabies virus do correlate to survival. Allowing a rabies antibody titer of >0.5 IU/mL to substitute for routine booster vaccination is safer for pets and more protective than current vaccine status alone.

Name: Doug Eckery

PP19

Email: douglas.c.eckery@aphis.usda.gov

Session: Immunology and Vaccines

Country: United States

Title: Fertility control for the management of free-roaming dogs: research update

Authors: Doug Eckery[1], Darcy Mora[1], Kathleen Eddy[2], Ruth Steinberger[3], Bill Clay[4], Charles Helwig[3], Scott Bender[5]

Affiliations: [1] US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA; [2] Colorado State University, Department of Biomedical Sciences, Fort Collins, Colorado, USA; [3] Spay FIRST!, Oklahoma City, Oklahoma, USA; [4] US Department of Agriculture, Wildlife Services, Deputy Administrator's Office, Washington, DC, USA; [5] The Navajo Nation, Veterinary Program, Chinle, Arizona, USA

Abstract: Overabundant feral dog populations are a large concern worldwide; they create several problems including transmission of disease to humans and livestock, predation of livestock, traffic accidents, nuisance behavior, and numerous bites. A common method of attempting to control these dogs has been through culling, sometimes as often as three times a year in some locations. The use of fertility control as a tool to aid in management strategies is considered to have numerous benefits and has attracted substantial attention. Moreover, modeling has shown that fertility control, in conjunction with vaccination against disease, can help reduce the transmission of zoonotic diseases and decrease the time required for disease eradication. However, to be successful a large proportion of the dog population must be treated. Spay/neuter clinics have been utilized with varying results, but are relatively expensive and are limited in the number of dogs that can be treated. Chemical contraception/sterilization could offer a humane, effective and less expensive alternative to surgical sterilization, and make it more feasible to target the large proportion of animals necessary. At the USDA, National Wildlife Research Center efforts are ongoing towards development of a contraceptive vaccine for use in free-roaming dogs. Research is also being conducted towards development of direct acting reagents or chemosterilants that are able to cause permanent sterility. The goal is to produce a reagent(s) that can cause permanent sterility after a single dose - one hit, permanent sterility. An update on progress to date in these areas of research will be reported.

Name: Elaine Fernandes

PP20

Email: elaineraniero@usp.br

Session: Immunology and Vaccines

Country: Brazil

Title: Comparative evaluation in situ of the immune response Th17 profile in cases of human rabies transmitted by dog and vampire bats

Authors: ER Fernandes[1], SM Achkar[1], MIS Duarte[2], F Guedes[1]

Affiliations: [1] Pasteur Institute of São Paulo, São Paulo, Brazil; [2] University of Sao Paulo, Faculty of Medicine, Department of Pathology, Sao Paulo, SP, Brazil

Abstract: The differentiation of Th17 cells from naive CD4+T cells is regulated directly by cytokines, mainly TGF-β, IL-6, IL-17 and transcriptional factors, as ROR-γ gamma t, ROR-α alpha, STAT3 and indirectly by other immune cells. Nevertheless, the evidence for the involvement in any central nervous system (CNS) infection is rare. So the aim of this work was to perform a comparative study by of the evaluation of cells expressing TGF-β, IL-6, IL-17; the rabies viral antigen and TCD4 lymphocytes in eight samples of CNS from human rabies cases, being four transmitted by dogs and four by vampire bats. Were selected fragments of cortex, hippocampus, basal ganglia, cerebellum and brainstem. To evaluate the expression of cytokines TGF-β, IL-6, IL-17, rabies viral antigen and TCD4 lymphocytes was performed the immunohistochemistry technique. All immunostained cells were quantified using a grid-scale in an area of 0.0625 mm2 considering 40 fields in each fragment of the CNS. Results were expressed in number of cells per mm2. Statistical analysis was carried out through the use of non-parametric test of Mann-Whitney with significance level of $p<0.05$. In relation to the viral antigen was observed an increase significant in cases of rabies transmitted by dog ($p<0.0001$). The cytokines expression as well the number of TCD4 lymphocytes was similar in two groups. Although there was no significant difference regarding the cytokines, in cases of human rabies transmitted by dog was observed a greater expression of TGF- β and IL-6. We could to speculate that in these cases the infection by a rabies virus variant more pathogenic, could lead specially to a Th17 profile immune response in comparison with the human rabies transmitted by vampire bats.

Name: Adriele Gregório

PP21

Email: hruthner@yahoo.com.br

Session: Immunology and Vaccines

Country: Brazil

Title: Activity of Dalbergia variabilis against genetic lineages from bats of rabies virus

Authors: ANF Gregório[1], MES Fernandes[1], AC Rodrigues[1], ACR Silva[1], TCP Souza[1], JGC Kawai[1], JA Montanha[2], PM Roehe[3], HBCR Batista[1]

Affiliations: [1] Pasteur Institute of São Paulo, São Paulo, Brazil; [2] Federal University of Rio Grande do Sul, Faculty of Pharmacy, Department of Production and Raw Material, Porto Alegre, Rio Grande do Sul, Brazil; [3] Federal University of Rio Grande do Sul, Institute of Basic Sciences of Health, Porto Alegre, Rio Grande do Sul, Brazil

Abstract: Hematophagous bat *Desmodus rotundus* is the main reservoir of wild rabies in Latin America but usually different species of insectivorous bats have been found infected with rabies virus (RABV). Despite the fatal aspect of rabies, two cases of cure of the disease in humans were recorded. In both of these cases, RABV was transmitted by bats, the record of these cases increased the researches about agents that could act as antiviral in rabies infection. The aim of this work was to evaluate an antiviral activity of plant extract *Dalbergia variabilis Vogel* against RABV isolated from bats. To this end, two strains of RABV were selected; one with genetic lineage characteristic of insectivorous bat *Eptesicus furinalis* and another with genetic lineage characteristic of hematophagous bat *Desmodus rotundus*. The RABV strains were titrated in presence and absence of a fixed concentration of hydroethanolic extract of *Dalbergia variabilis Vogel* (6,25mg/mL, as determined by Maximum Tolerated Concentration- MTC assay), in 96-well microplates with N2a cells. After 3 days, plates were fixed and stained by Direct Immunofluorescence. Viral titres were calculated according to the Reed and Muench method and expressed in Tissue Culture Infective Doses (TCID50). For both RABV strains tested the reduction of viral titre was 2.50 log10 TCID50 showing antiviral activity of *Dalbergia variabilis* against RABV. Our results show that the *Dalbergia variabilis Vogel* is effectiveness against RABV with genetic lineages of hematophagous and insectivorous bats.

Name: Darryn Knobel

PP22

Email: dknobel@rossvet.edu.kn

Session: Immunology and Vaccines

Country: St Kitts & Nevis

Title: Do animal rabies vaccines have a nonspecific protective effect when administered to young puppies in high-mortality settings?

Authors: Darryn Knobel[1], Anne Conan[1], Greg Simpson[2], Bjorn Reininghaus[3]

Affiliations: [1] Ross University School of Veterinary Medicine, Center for Conservation Medicine and Ecosystem Health, Basseterre, St Kitts; [2] University of Pretoria, Faculty of Veterinary Studies, Centre for Veterinary Wildlife Studies, Pretoria, South Africa; [3] Mpumalanga Veterinary Services, Department of Agriculture, Rural Development, Land and Environmental Affairs, Thulamahashe, South Africa

Abstract: There is growing evidence from laboratory and field trials that vaccination of dogs younger than three months with high-quality inactivated animal rabies vaccines is effective. World Health Organization guidelines recommend that all dogs, including puppies less than three months of age, are vaccinated during mass vaccination campaigns. Here, we present preliminary evidence that these vaccines may have a nonspecific protective effect in this age group in high-mortality settings (i.e. enhance survival to diseases other than rabies). We implemented a health and demographic surveillance system in dogs that monitored the entire owned dog population within a defined geographic area in a rural community in Mpumalanga Province, South Africa. We quantified demographic rates among owned dogs over a 24-month period, from 1st January 2012 through 1st January 2014, and assessed their implications for rabies control. Crude annual mortality rates per 1,000 dog-years of observation (dyo) were 406 in 2012 and 568 in 2013, with extremely high age-specific death rates in puppies 0-3 months old. We fitted a Poisson regression model to assess the effects of sex, age class and vaccination status on mortality rates. In the age class 0-3 months, the mortality rate of dogs vaccinated against rabies was significantly lower than that of unvaccinated dogs (2012: mortality rate ratio [MRR] = 0.11, 95% CI = 0.05-0.21; 2013: MRR = 0.31, 95% CI = 0.11-0.69). We consider possible causes for this apparent association, arguing that the reduction in mortality is unlikely to be due to the specific protective effect of rabies vaccination, given the low incidence of the disease in the population during the period in question, particularly among the age group concerned. We conclude with a discussion of the implications this finding may have for dog rabies control in resource-limited settings.

Name: Susan Moore

PP24

Email: smoore@vet.ksu.edu

Session: Immunology and Vaccines

Country: United States

Title: Serologic detection of equine antibodies to vaccine and field strains of rabies virus using a multiplex microsphere-based assay

Authors: Susan Moore[1], Kaitlin Haukos[2], Kelley Black[2], Elizabeth Davis[3], Melinda Wilkerson[2]

Affiliations: [1] Kansas State University Rabies Laboratory, Manhattan, Kansas, USA; [2] Kansas State University, College of Veterinary Medicine, Department of Diagnostic Medicine/Pathobiology, Manhattan, Kansas, USA; [3] Kansas State University, College of Veterinary Medicine, Department of Clinical Sciences, Manhattan, Kansas, USA

Abstract: Rabies is a fatal neurological disease caused by an RNA virus in the family Rhabdoviridae. Horses are susceptible to rabid wildlife. The majority of viral strains isolated from rabies positive horses in Kansas are skunk; however several bat strains have also been identified. The hypothesis that horses vaccinated with laboratory adapted rabies strains have weaker antibody titers to virus variants occurring in nature was investigated. A multiplex-bead-based indirect immunoassay was developed to screen sera obtained from adult horses against rabies antigens isolated from seven rabies virus isolates. This multi-analyte technology (xMap) was designed to quantify equine IgG binding to viral antigens derived from the seven different rabies virus strains, simultaneously. Characterization of the dominant viral proteins in the antigen preparations was performed by silver stain of SDS-PAGE. Coupling of G and N viral proteins derived from three laboratory rabies strains to three sets of xMag beads was confirmed. The 7-plex set of rabies antigen coated xMap beads was tested against serum samples obtained from 18 horses before and after rabies vaccination. Results were compared to ELISA and RFFIT results from the same serum samples. Analysis of the antibody response measurements indicate that all horses increased their vaccine response following vaccination, but a variety of responses were detected to rabies strains that differ from the vaccine strain.

Name: Alejandro Parola

PP25

Email: aparola@fundacioncassara.org.ar

Session: Immunology and Vaccines

Country: Argentina

Title: Pilot lot production of a chromatographically purified vero cell rabies vaccine

Authors: Carlos Palacios[1], Santiago Chiappini[1], Analía De Nichilo[1], Ezequiel Nuguer[1], Oscar P Larghi[2], Alejandro Daniel Parola[1,3]

Affiliations: 1 Pablo Cassará Foundation, Buenos Aires, Argentina; 2 Pablo Cassará Laboratory, Buenos Aires, Argentina; 3 Institute of Science and Technology "Dr. César Milstein" – CONICET, Buenos Aires, Argentina

Abstract: Rabies vaccine production in Latin America is still based on nerve tissue substrate (NTV), it is well known that these vaccines are not safe inducing adverse reactions, and are less immunogenic than cell culture derived vaccines. More than three decades ago, WHO has recommended NTVs discontinuation. In this regard, in a previous report, we showed the development of a chromatographically purified Vero cell rabies vaccine (CPRV), describing generation and characterization of viral and cell banks, viral production in spinner flasks and a purification process involving different chromatographic steps. The aim of this study is to scale up both upstream and downstream steps of a CPRV production. In this research, Vero cells were grown in a five-liter stirred tank bioreactor (BIOSTAT Aplus, Sartorius Stedim Biotech) using Cytodex 1 microcarriers. The cell culture was started by seeding 0.20-0.25 x 106 cells/ml in M199 cell culture medium, and infected at a MOI of 0.1 ID50/ml once cell density reached levels between 2,5 and 3,5 x 106 cells/ml. Harvests were taken every 48 h, during at least 14 days post infection. Cell culture was monitored for cell density, glucose and lactate. Viral production was followed by glycoprotein content and viral titer determination. Cells were also observed by immunofluorescence to check viral infection. The described process yielded a volumetric productivity of 2,500 glycoprotein IU/L, potentially reaching 11,000 vaccine doses without considering loss during downstream. Additionally, partially purified and β-propiolactone inactivated virus showed protective levels in NIH assay, demonstrating that the obtained antigen induces a specific immune response. Finally, the purification process involved clarification using depth filters, concentration by ultrafiltration/diafiltration, and two chromatographic steps (cation exchange followed by gel filtration). The purified and inactivated viral particles complied with European Pharmacopoeia requirements.

Name: Dmitry Volokhov

PP26

Email: dmitriy.volokhov@fda.hhs.gov

Session: Immunology and Vaccines

Country: United States

Title: Experimental anti-rabies virus immunization of laboratory animals with mRNA constructs encoding the rabies virus glycoprotein gene and with plant-derived rabies virus purified glycoprotein

Authors: DV Volokhov[1], V Furtak[1], Y Kleschenko[1], A Dabrazhynetskaya[1], S Pritchard[2], RM Jones[2], V Yusibov[2], VE Chizhikov[1]

Affiliations: [1] Food and Drug Administration, Center for Biologics Evaluation and Research, Rockville, Maryland, USA; [2] Fraunhofer USA, Center for Molecular Biotechnology, Newark, Delaware, USA

Abstract: Immunization of animals with recombinant plant-derived antigens or mRNA encoding viral antigens has shown to be efficient approaches to induce a protective immunity against different viral infections. Advantages of mRNA-based and plant-derived vaccine candidates include (i) the simplicity of their construction, (ii) the low manufacturing cost, and (iii) the ability to elicit adequate immune response to target antigens. The goal of this study was to assess the immunogenicity and protectivity of anti-rabies mRNA-based and plant-derived vaccine candidates in rabbit and guinea pig models. The plant-derived rabies virus glycoprotein (GPΔTMD) was expressed in the wild type non-genetically modified of *Nicotiana benthamiana* in the truncated GP form (without transmembrane and cytoplasmic protein domains). The chromatographically purified protein (≥90% by SDS-PAGE) was used for immunization experiments. mRNA encoding the GP gene was synthetized using the T7 mScript Standard mRNA Production System. The mRNA was purified, tested for the absence endotoxins, and used for immunization experiments. Analysis of antibodies elicited in animals immunized with GPΔTMD revealed very low titers of anti-rabies neutralizing antibodies (0.1-5 UI/mL; measured by RFFIT) but high titers of anti GP antibodies (≥ 1:400,000; detected in ELISA). However, all animals that received three IM doses (100 µg per dose) of the GPΔTMD with Emulsigen-D adjuvant were protected against IM challenge with CVS-11 virus (at dose of 6x106 FFU). Immunization of animals with naked mRNA resulted in production of variable titers of anti-rabies neutralizing antibodies (0.1-60 IU/mL). Nevertheless, all animals that received three IM doses (50 or 200 µg mRNA per dose) were protected against IM CVS-11 challenge. Our results demonstrated that the use of mRNA and plant-derived glycoprotein as alternative vaccine candidates could be a promising approach for anti-rabies immunization in animals and possibly in humans.

Name: Dmitry Volokhov

PP27

Email: dmitriy.volokhov@fda.hhs.gov

Session: Immunology and Vaccines

Country: United States

Title: Evaluation of Micronjet600 microneedles for intradermal anti-rabies immunization with experimental DNA-based vaccine and with RabAvert human rabies vaccine in rabbit model – the dose-sparing study

Authors: DV Volokhov[1], V Furtak[1], E Kochba[2], VE Chizhikov[1]

Affiliations: [1] Food and Drug Administration, Center for Biologics Evaluation and Research, Rockville, Maryland, USA; [2] NanoPass Technologies Ltd., Nes Ziona, Israel

Abstract: Intradermal delivery of different human vaccines has been shown to result in dose sparing. In our study we evaluated the ability of reduced doses of the intradermally delivered commercial inactivated rabies vaccine for human use (RabAvert) and our experimental DNA-based vaccine to induce sufficient amount of anti-rabies neutralizing antibodies in rabbits in comparison to that elicited using the regular intramuscular route. To demonstrate a dose-dependent response (dose-sparing effect) with RabAvert and our DNA vaccine using MicronJet600 microneedles, rabbits were immunized with different doses of each vaccine on the days 0, 7, and 28. The serum samples were collected on 14th and 42nd days after immunization and used to assess the titers of anti-rabies neutralizing antibodies in the RFFIT. The results of the study demonstrated that the intradermal delivery leads to the clear dose-sparing effect for both tested vaccines in the rabbit model; and potentially, this approach can be used in order to reduce the overall rabies vaccination associated expenses for animals or humans.

Name: Ruth Steinberger

PP29

Email: ruth@spayfirst.org

Session: Rabies in Domestic Animals

Country: United States

Title: Creating meaningful goals to face the challenges of providing dog population management services on Tribal Lands in the US

Authors: Kimberly D Carter[1] and Ruth Steinberger[2]

Affiliations: [1] Oklahoma State University, College of Veterinary Medicine, Shelter Medicine and Junior Surgery, Stillwater, Oklahoma, USA; [2] Spay FIRST!, Oklahoma City, Oklahoma, USA

Abstract: The management of dog populations on Native American reservations presents challenges that are reflected in excessive numbers of bites and animal welfare issues. Reducing the number of unwanted dogs requires high volume sterilization services. Effective spay/neuter/vaccination services at First Nations are very costly and difficult to operate. Non-surgical options are needed. In order to develop a non-surgical sterilization option for free roaming female canines, Spay FIRST partnered with USDA/NWRC to conduct testing of GonaCon, a GnRH antigen. A field study was approved by the EPA and a project in partnership with a northern tribe was instituted. GonaCon, rabies vaccine and parasite treatment were included. Participation at all points of contact was heavily incentivized. Nineteen female dogs were injected with GonaCon in November 2014. Protocol included collection of serum six and 12 months post-injection. In May 2015, attempts were made to contact the caregivers for follow up protocol in June. Six of the nineteen dogs were deceased, four caregivers were unreachable. Commitments were made by caregivers to present eight of the remaining nine dogs for surgery. Four of eight dogs arrived. Some dogs were believed to have gone into estrus; one had delivered live puppies. However no caregiver followed protocol to notify of suspected vaccine failure and make subsequent arrangements for surgery. The spay/neuter clinic was held on the reservation. To reach this remote site, three round trip flights were booked for the principal investigator and surgeon. Due to weather and airline problems, the veterinarians missed connecting flights both going and returning, incurring significant expenses. The vehicle with equipment and supplies traveled 2618 miles round trip. The obstacles to completion of this field trial highlight the need for development of non-surgical options in penned studies, toward the goal of deepening involvement of tribal and federal health agencies in dog population management programs.

Name: Maria Luiza Carrieri

PP30

Email: mlcarrieri@gmail.com

Session: Wildlife Rabies

Country: Brazil

Title: Rabies in wildlife in South America

Authors: Maria Luiza Carrieri[1], Rosely Cerqueira de Oliveira[2], Ivanete Kotait[3]

Affiliations: [1] Butantan Institute, Rabies Section, São Paulo, Brazil; [2] Brasilia Academy of Medicine, Brasilia, Brazil; [3] Vencofarma Laboratory of Brazil, Londrina, Paraná State, Brazil

Abstract: After years of investments from South America countries to control canine rabies (variants 1 and 2), with satisfactory results, through vaccination and different prophylactics measures, another challenge with greater difficulty arises in the region: wildlife rabies. Several genera and/or species of Carnivora, Chiroptera and Primate already emerge as important reservoirs of rabies distinct antigenic and genetic variants and are also seen serious threats that require further study, investment in methods of control and use of oral vaccines, such as developed countries have sought to rabies control. The purpose of this summary is to describe the gender and/or most important species of which were detected viruses or antirabies antibodies. Among the terrestrial wild stand out *Urocyon cinereoargenteus*, *Cerdocyon thous*, *Pseudalopex vetulus*, *Chrysocion brachyurus*, *Leopardus tigrinus*, *Dusicyon gymnocercus* and *Conepatus chinga*. A feature of these reservoirs and cycles between these animals is the close relationship between the variant detected in dogs and these above mentioned species. Another important reservoir is the *Callithrix jacchus*, that have the more distinct variant in the region. Among the bats, rabies has been detected in about 55 species, especially *Desmodus rotundus* (across the region), *Tadarida brasiliensis*, different species of *Artibeus* sp genera, *Lasiurus* sp, *Myotis* sp, *Histiotus* sp, *Eptesicus* sp. Rabies in insectivorous bats is characterized by high diversity of viral variants, the co-circulation and inter-species transmission. Importantly, the laboratory techniques deployed in most countries for surveillance of rabies in South America, were essential to start programs, although the importance of this is very underestimated. Recognition and identification of wild species will allow better use of resources in public health. It's imperious that health services in South America starting field work, to making good use of an experience in North America and Europe.

Name: Dylan Ray

PP31

Email: Tad.Theimer@nau.edu

Session: Wildlife Rabies

Country: United States

Title: Identifying individual striped skunks using pelage patterns

Authors: Dylan Ray[1], Tad Theimer[1], David Bergman[2]

Affiliations: [1] Northern Arizona University, Flagstaff, Arizona, USA; [2] US Department of Agriculture, Wildlife Services, Arizona, USA

Abstract: Recognizing individual animals is critical for understanding many aspects of animal ecology and behavior, including estimating population size and the potential for disease spread, but individually marking animals can be costly in both time and money. Some species have enough variation in naturally-occurring markings that individuals can be recognized without adding artificial marks, tags or collars. We tested 1) whether striped skunks in Flagstaff, Arizona could be reliably recognized based on naturally-occurring color patterns and 2) whether camera trap position influenced how effective that technique was under field conditions. To answer the first question, we asked volunteers to estimate the number of skunks they could distinguish from an array of 28 photographs taken of striped skunks anesthetized and captured for other studies. To answer the second question, we placed 2 trail cameras at each of three winter den locations and photographed skunks either with the camera oriented vertically 30cm above ground so that photographs were taken from the side, or with cameras mounted 2 meters above the den entrance to capture photos from above. We then asked volunteers to estimate how many individuals they could recognize from a subset of 24 photos. We found that 1) volunteers reliably identified individual skunks based on coat color variation and 2) field identification was significantly better when cameras were mounted to capture photos from above. We demonstrated that natural variation can be used to recognize individual striped skunks if cameras are correctly positioned, and this should facilitate future studies of behavior, ecology, population dynamics and disease management.

Name: Danielle Araujo

PP32

Email: daniellebastos@yahoo.com.br

Session: Wildlife Rabies

Country: Brazil

Title: Training of agents of endemics diseases for rabies surveillance in terrestrial wildlife from Ceara State, northeast of Brazil

Authors: Danielle Bastos Araujo[1], Naylê Francelino Holanda Duarte[2], José Airton de Negreiros[3], Luiz Francisco Sanfilippo[1], Rogério Azevedo[3], Keila Iamamoto Nogi[4], Graciane Capolare Medeiros[4], Luzia Alves Martorelli[5], Roberta de Paula Oliveira[6], Júlio César Augusto Pompei[7], Otorino Cosivi[4], Mônica Martini[4], Benedito Neilson Rolim[8], Marcia Chame[9], Edison Luis Durigon[1], Silvana Regina Favoretto[1,4]

Affiliations: [1] University of São Paulo, Laboratory of Clinical and Molecular Virology, São Paulo, SP, Brazil; [2] Center of Vector-borne Diseases, Health Department of Ceara State (NUVET-SESA), Fortaleza, Ceara, Brazil; [3] Regional Coordination of Health, Tiangua, Ceara, Brazil; [4] Pasteur Institute of São Paulo, SP, Brazil; [5] Center of Zoonosis Control, São Paulo, SP, Brazil; [6] Health Department of Ceara State (SESA), Fortaleza, Ceara, Brazil; [7] PANAFTOSA, Panamerican Health Organization/World Health Organization (PAHO/WHO), Duque de Caxias, Rio de Janeiro, Brazil; [8] Central Laboratory of Public Health (LACEN), Fortaleza, Ceara, Brazil; [9] Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, RJ, Brazil

Abstract: Although the rabies virus variant maintained by dogs is controlled in most parts of Brazil, the variants found in terrestrial wildlife are an issue in the Northeast region, especially in Ceara State. In the last two years, 21 animals of these species (mainly crab eating foxes [*Cerdocyon thous*] and marmosets [*Callithrix jacchus*]) were diagnosed as positive. With the objective of provide information regarding the ecoepidemiology of rabies in terrestrial wildlife from Ceará, a collaborative project is being conducted to permit the development of more efficient measures of rabies surveillance and control. The first phase of the project is already implemented and consisted in the equipment and training of agents of endemic diseases – employees working in close contact with the local population of urban and rural areas - in 10 cities with the majority of cases, for the collection of central nervous system samples from wild animals found dead. This type of passive surveillance is already done, but with the training we have the guaranty that the procedures will result in samples collected with the ideals conditions for virus diagnosis and characterization and also that all the biosafety requirements are observed. As a result of the training, the agents are highly motivated, not only for the passive but also for the active surveillance through the identification of areas where wild animals are in close contact with humans and domestic animals. In these areas, our study is capturing animals to collect blood samples for antibodies detection, obtaining georeferenced information about the environmental characteristics and applying a survey with the local population. This study of the interaction between humans, wild and domestic animals and the environment will provide parameters for the first pilot project of wildlife vaccination aimed for this region and important information about the ecoepidemiology of terrestrial wildlife rabies in Brazil.

Name: Dennis Donovan

PP33

Email: dennis.donovan@ontario.ca

Session: Oral Rabies Vaccine and Rabies Control

Country: Canada

Title: ONRAB - immune response and protection in raccoons

Authors: LJ Brown[1], RC Rosatte[1], C Fehlner-Gardiner[2], DDonovan[1], JC Davies[1]

Affiliations: [1] Ontario Ministry of Natural Resources, Wildlife Research and Monitoring Section, Peterborough, Ontario, Canada; [2] Canadian Food Inspection Agency, Ottawa Laboratory-Fallowfield, Ottawa, Ontario, Canada

Abstract: We investigated the immune response and protection conferred in raccoons (*Procyon lotor*) following consumption of ONRAB oral rabies vaccine baits. Forty-two wild-caught, captive raccoons were each offered an ONRAB vaccine bait; 21 controls received no vaccine baits. Blood samples collected from all raccoons before treatment, and each week posttreatment for 16 wk, were assessed for the presence of rabies virus antibody. In the bait group, an individual was considered to have responded to vaccination if serum samples from three or more consecutive weeks were antibody-positive. Using this criterion, 77% (20/26) of raccoons that consumed ONRAB baits with no observed vaccine spillage (full dose) demonstrated a humoral immune response. In the group that received a partial dose (0.05–0.90 mL vaccine recovered), 50% (8/16) of raccoons responded to vaccination. Regardless of the vaccine dose received, among the 28 raccoons that responded to vaccination 18 had antibody initially detectable at week 2 and 22 remained antibody-positive for at least 10 consecutive weeks. Kinetics of the humoral immune r8esponse suggest that the best time to conduct postbaiting surveillance for evidence of vaccination would be 6–13 wk following bait deployment, with the highest antibody prevalence expected between weeks 8–10. A sub-sample of 29 raccoons (20 ONRAB, 9 controls) was challenged with raccoon rabies virus variant 350 days posttreatment. Eight of nine controls (89%) developed rabies whereas 15/20 vaccinates (75%) survived. Survival following rabies challenge was significantly higher in raccoons presented ONRAB vaccine baits.

Name: Camila Appolinario

PP34

Email: jane@fmvz.unesp.br

Session: Bats and Lyssavirus: Host and Virus Diversity

Country: Brazil

Title: Re-emergence of bovine rabies after 28 years in the Farm School of FMVZ-UNESP, Botucatu, Brazil

Authors: Fernando FC Castro[1], Camila M Appolinario[1], Bruna D Ribeiro[1], Vanessa Pelicia[1], Clovis R Fonseca[1], Gisely T Barone[2], Luzia F Martorelli[2], Matheus R Mioni[1], Jane Megid[1]

Affiliations: [1] São Paulo State University, Faculty of Veterinary Medicine and Animal Science ("Julio de Mesquita Filho"), Botucatu, SP, Brazil; [2] Center of Zoonosis Control, São Paulo, SP, Brazil

Abstract: The Edgardia farm, property of the Faculdade de Medicina Veterinária e Zootecnia (UNESP) is located in Botucatu city (São Paulo-Brazil; (22° 49´ 0127" S, 48 ° 24´ 901" W). This farm is divided into several areas in which cattle, sheep, horses, pigs, poultry and buffaloes are raised. It is known that in the farm's area there are some shelters of the common hematophagous bats (*Desmodus rotundus*). Two of these roosts have been monitored during years for biological behavior studies and populational sizing/characterization for educational proposals. Although attacks to the horses and cows were frequently observed at Edgardia farm no occurrence of rabies cases in domestic animals in the last 28 years were reported. Considering the low epidemiological risk anti-rabies vaccination in those animals was no longer recommended. On March 3, 2015, a male bovine, Nelore started to present neurological signs and died within 3 days (March, 6) resulting positive for rabies by FAT and intracerebral inoculation in mice. In March 18, a horse died and was also diagnosed with rabies. Considering these events all the ruminants and equines were vaccinated against rabies on April, 10 and revaccinated in May, 11. Despite vaccination other 2 cases of rabies were diagnosed, one in a horse in May 1st (after first dose of vaccine) and another in a bovine in June 1st (after revaccination). Those last 2 rabid animals were maintained in the same place which is 200 meters distant of a hematophagous bat shelter. Cicatricial lesion due bat bites was observed in the back region of one of the rabid animals. Most part of the Edgardia farm land is characterized by a well preserved forest area with two bats shelters in hollow trees identified by farm's workers. It is known that bats located in the property are typically yellow. After these rabies cases mist nets were used to capture bats foraging at this farm and the number of brown furred bats were increased drastically (up to 40%) in 2015. A relevant aspect observed was a reduction in the yellow bat shelter's colony compared to the previous counter realized before the beginning of these rabies cases in March. Serology test was performed in yellow and brown furred bats capture in May, 2015, totalizing 23 individuals. Among the yellow bats 8 out 11 were considered serologically positive while among brown bats 5 out 12. The reason for the increase in the number of brown bats in the farm, where previously there was almost only yellow bats, is still unknown but we assume that brown bats may have been displaced from neighboring municipalities where outbreaks of rabies in herbivores had been recorded and can be responsible for the introduction of rabies virus in Edgardia's shelters. Ecological changes, the reduction in bats control measures associated with the absence of vaccination are factors that can be involved in the reemergence of rabies in this region.

Name: Camila Appolinario

PP35

Email: jane@fmvz.unesp.br

Session: Bats and Lyssavirus: Host and Virus Diversity

Country: Brazil

Title: Seropositivity for rabies in vampire bats captured in a rabies outbreak area in Botucatu, SP Brazil

Fernando FC Castro[1], Camila M Appolinario[1], Bruna D Ribeiro[1], Vanessa Pelicia[1], Clovis R Fonseca[1], Gisely T Barone[2], Luzia F Martorelli[2], Matheus R Mioni[1], Jane Megid[1]

Affiliations: [1] São Paulo State University, Faculty of Veterinary Medicine and Animal Science ("Julio de Mesquita Filho"), Botucatu, SP, Brazil; [2] Center of Zoonosis Control, São Paulo, SP, Brazil

Abstract: From March 2015 four cases of rabies were diagnosed in production animals (2 horses and 2 cattle) on the school farm belonging to the Faculdade de Medicina Veterinaria e Zootecnia (FMVZ-UNESP) in Botucatu. The presence of shelters and the occurrence of vampire bat (*Desmodus rotundus*) attacks were an ordinary event in the property but without rabies reports in the last 28 years. Considering these cases of rabies the main objective of the study was to evaluate the viral circulation and the presence of positive bats in the two vampire bat shelters previously identified in the farm. The first shelter was located on a tree whole (position K22 765326.42 E 7479180.05), composed by single males, brown coat and yellow coat. The second shelter was located in a focal point of a tree near a citrus crop (position K22 7656448.14 E 7474603.20), with a population estimated in 30-50 vampire bats, composed by males and females of different ages and coat color (brown or yellow). Captures were performed in April, 2015 with two mist nets installed strategically out of the shelters. In first 5 *Desmodus rotundus* were caught and in the the second this number increased to *18 Desmodus rotundus*. During the capture data such as age, gender and reproductive status were noted. Animals were individually identified and transported to Dpt. of Veterinary Hygiene and Public Health of FMVZ-UNESP, where they were anesthetized with isoflurane, submitted to drawn blood and euthanized with intraperitoneal ketamine when brain and salivary glands were collected for rabies virus detection using real-time PCR and Fluorescent Antibody Test (FAT). Sera were used in anti-rabies serology through the Simplified Inhibition of Fluorescent Focus Test (SFIMT). Of the 23 captured animals 16 were males, 7 were females, 11 were yellow, 12 were brown, 20 were adults and 3 were young, being the age classification done according to the degree of ossification in the long bones of the wing. None of the salivary glands or brains of these vampire bats resulted positive for the presence of rabies virus in FAT or real-time PCR. For anti-rabies serology 13 out 23 presented antibodies levels equal or higher than 0.5 I.U/ ml, being 10 males and 3 females. Although not statistically significant a greater number of seropositive males is associated with a higher risk of contamination of the gender explained by the constant migration and diffusion from the time of weaning, coexistence with other males and fights for dominance. The prevalence of adult seropositives indicates that adult animals are more exposed to the virus by their displacement.

Name: Ellen Elizabeth Laurindo

PP36

Email: ellenlaurindo@hotmail.com

Session: Bats and Lyssavirus: Host and Virus Diversity

Country: Brazil

Title: Analysis of bats captured in the state of Paraná (Brazil) between 2009 and 2014: species, location of capture, behavior

Authors: Jéssica Giordana de Pádua Formigoni, Ivan Roque de Barros Filho, Ellen Elizabeth Laurindo[1], Ana Pérola Drulla Brandão, Alexander Welker Biondo, Elaine Fátima de Sena[1]

Affiliations: [1] Department of Animal Health, Ministry of Agriculture, Livestock and Food Supply, Brasilia, Brazil

Abstract: Bats are one of the most diversified group of mammals in the world, representing 22% of known mammalian species. These animals play an important role in the equilibrium of ecosystems, but its presence may result in undesirable interactions with humans and your pets, increasing the risk of transmitting diseases such as rabies, if the bat is infected. This study aimed to describe the species caught by the service of Paraná Agricultural Protection Agency (Adapar) in rural and urban areas of Paraná State, Brazil, from 2009 to 2014. They were captured 478 bats of which 78.2% were found in altered behavioral conditions (passive surveillance) and 21.8% in shelters review (active surveillance). Species identification was performed in 91.2% of bats submitted to the laboratory, belonging to 26 species in three families (*Phyllostomidae*, *Vespertilionidae* and *Molossidae*). In urban areas there was great dominance *Molossus* spp with 53.2%, followed by *Lasiurus* spp with 6.36% and *Artibeus* spp with 5.51% of subjects. The bats were captured in places like backyards, residential interior (in urban and rural areas), public roads, schools, churches, health centers, hospitals, shops and abandoned buildings. Municipalities which were registered more shots of bats were Guarapuava (11.74%), Curitiba (5.66%) and Jacarezinho (4.61%). Most species was insectivorous and *Molossus* spp species and common vampire bat, predominated during the study period.

Name: Alejandro Zaldivar Gomez

PP38

Email: al3xvet@gmail.com

Session: Epidemiology and Modeling

Country: Mexico

Title: Canine aggression incidence in four municipalities of State of Mexico: a spatio-temporal approach

Authors: A Zaldivar-Gomez[1], JA Hernandez-Magaña[2], C Delgado-Urbina[1]

Affiliations: [1] State Public Health Laboratory of the State of Mexico, Toluca, Mexico; [2] Zoonoses and Vectors Department, Toluca, Mexico

Abstract: Dog bites in human beings are an important public health problem, as much for the injuries they cause, as for the possibility of transmission of infectious diseases such as rabies, the psychological consequences, incapacity and the economic costs derived from medical treatments. A retrospective study was conducted using information on cases of canine attacks reported in 2013 in four municipalities of State of Mexico, Mexico. In addition, a database with the geographic location of all health care units and their monthly reports on dog bites during the period 2008-2013 was developed in order to learn the cumulative incidence. This data was spatialized using the kernel density estimation to develop a spatio-temporal dissemination model of the annual incidence of attacks. During the period 2008-2013, 5,705 canine attacks were recorded in the study area. In 2013 alone, there were 986 filed cases. Cumulative incidence of canine aggression was 0.5% per year (95% CI: 0.1-1.2) per 1000 inhabitants. During 2013, the majority of victims of attacks were children between 6-5 year-olds (25.5%). More injuries were present in men (53.7%), and the largest number of injuries (30.4%) took place during the summer months. In 55.8% of cases of dog attacks affected the victim's lower limbs and 11% the head and neck. In distribution terms, the kernel density map showed that the highest incidence of attacks occurred in areas with a high human population density, poverty and marginalization where the presence of stray dogs was a constant factor and the generation of organic waste matter ensures feeding and proliferation. The data obtained showed that in the study area, canine attacks affected the health of the population persistently. Therefore, it is important to note that studies based on the population must be carried out to assess the nature and prevalence of canine aggression.

Name: Elaine Fatima de Sena

PP39

Email: elaine.sena@agricultura.gov.br

Session: Epidemiology and Modeling

Country: Brazil

Title: The surveillance of nervous syndrome in herbivores and rural rabies in Brazil

Authors: Carlos Henrique Pizarro Borges[1], Elaine Fatima de Sena[1], Gustavo Gomes Fonseca[2], Mariana Figueira Dornelas[2]

Affiliations: [1] Department of Animal Health, Ministry of Agriculture, Livestock and Food Supply, Brasilia, Brazil; [2] Graduate Student in Veterinary Medicine at the University of Brasilia, Brazil

Abstract: The Brazilian surveillance in animal health aims at the investigation of diseases grouped by syndromes, not diseases isolated in order to increase the sensitivity. Four syndromes are the objective of this surveillance: vesicular, nervous in herbivores, hemorrhagic of swine and aviary respiratory/nervous. In the case of nervous syndrome in herbivores, the target diseases are: rabies, equine encephalitis and transmissible spongiform encephalopathies (TSE). Surveillance of nervous disease syndromes in herbivores in Brazil is mainly addressed to rabies, which is considered endemic in rural areas. In the map of areas of risk for rabies in the world (2012), of the World Health Organization (WHS), the entire Brazilian territory is presented as high-risk area. Since 1966, Brazil has been adopting specific measures for rabies in herbivores, and is currently the National Program for Control of Rabies in Herbivores, which aims to reduce the occurrence of the disease, through the following actions: epidemiological surveillance; strategic vaccination of susceptible species; health education; control of the transmitters of rural rabies. Due the importance of rabies, in Brazil it is mandatory to report to the Official Veterinary Service (OVS) any suspicion or occurrence of nervous diseases in herbivores, which will develop investigation and control activities, in order to preserve human and animal health. Between years 2010 to 2014, the Brazilian OVS recorded 4,441 rabies outbreaks in herbivores and were carried out 12,603 laboratory tests for rabies in these animals. In 2014, of 1,334 cases of rabies registered by OVS, about 90% occurred in cattle, 10% in horses and 1% in sheep. In Brazil, the main transmitter of rural rabies is the vampire bat Desmodus rotundus, widely distributed throughout the territory. These bats use as shelter caves, rifts and holes in trees and abandoned buildings. The great offer of these shelters and the significant growth of the herds in the country, currently around 200 million cattle, are the main contributing factors to the spread of rabies to herbivores, making unlikely the rural rabies eradication and requiring continuity of specific actions by official and private sectors.

Name: Christine Fehlner-Gardiner

PP40

Email: Christine.Fehlner-Gardiner@inspection.gc.ca

Session: Epidemiology and Modeling

Country: Canada

Title: Rabies in Canada - 2014

Authors: M Kimberly Knowles[1], Josephine Kush[2], Maria Harding[1], Kristi Bangs[1], Zaheer Iqbal[2], Christine Fehlner-Gardiner[1]

Affiliations: Canadian Food Inspection Agency, Canada: [1] Ottawa Laboratory-Fallowfield; [2] Lethbridge Laboratory

Abstract: In 2014, the CFIA laboratories tested 1919 samples for rabies, of which 93 (4.8%) were positive. The majority of samples were analysed in the fluorescent antibody test, with a small number tested by immunohistochemistry (n=4) or quantitative RT-PCR (n=4, human suspect cases). Bats accounted for the highest proportion of cases (50.5%), followed by skunks (23.7%) and arctic foxes (10.8%). Detection of wildlife virus variants in domestic species was similar to previous years, with cases detected in dogs (4), bovines (3), cats (2), and one horse, the result of skunk variant-virus in western Canada (livestock, one cat) or fox-variant virus in northern Canada (dogs). A cat rabies case from Quebec was due to a bat-variant virus. A fox-variant outbreak was detected in Labrador, and a new incursion of raccoon-variant was detected in New Brunswick; both outbreaks have continued into 2015 with 12 and 13 cases detected, respectively, in the first six months. Raccoon rabies was last detected in New Brunswick in May 2002, in the same county at the centre of the 2014/2015 outbreak. Since May 2012, only cases due to bat-variant rabies virus were detected in southwestern Ontario, allowing this region to declare freedom from both raccoon- and fox-variant rabies viruses. In April 2014 rabies management in Canada changed significantly, with many activities previously conducted by the federal government being assumed by provincial authorities. The submission of samples to the CFIA laboratories during this transition period varied depending on province, with an overall decrease of 44.6% from 2013. Whereas no significant change was observed for the Atlantic provinces, the northern Territories, Alberta or Saskatchewan, the provinces of Ontario, Quebec, British Columbia and Manitoba saw submission levels drop between 47% and 64%. As of 2015, these provinces now have established rabies programs, with the result of samples submissions beginning to return to expected levels.

Name: Maria Susana Moreno Fernandez

PP41

Email: susan_marly@hotmail.com

Session: Epidemiology and Modeling

Country: Mexico

Title: Epidemiology of rabies in Coahuila, Mexico

Authors: FMS Moreno, BAM Aguilar, GZL Carrales, AL Coronado

Affiliations: Coahuila State Laboratory of Public Health, Saltillo, Coahuila, Mexico

Abstract: The State of Coahuila presented problems because it did not have a laboratory for rabies diagnostics. The samples were sent to nearby laboratories, but many times the information was lost because the hired laboratory did not emit the results in an opportunistic manner. In 2010, the Rabies Laboratory of Coahuila starts working, solving the diagnostic inconveniences in the state and improving the epidemiologic surveillance, thus allowing opportunistic action. Materials and Methods: This study reports the total amount of samples analyzed since the creation of the laboratory until June 2015, as well as information of previous years as a background. In total, 3704 samples were analyzed: 3393 dogs, 281 cats, 16 chiropterans, 6 coyotes, 2 skunks, 2 rodents, 1 squirrel, 1 raccoon, 1 rabbit and 1 badger. Results: Positive cases were observed only in chiropterans, specifically from Piedras Negras, Cuatrociénegas and Saltillo. In none of the cases the antigenic variant was able to be determined, because there was an insufficient amount of sample received to fulfill the minimum requirements for its diagnostic and follow up at the InDRE. Observations: Low incidence of positive cases in the State, but there is still to consider that the surveillance in wildlife is scarce, due to the fact that it is difficult to access the areas where these live, also the lack of resource for the zoonosis personnel complicates monitoring, there is a lack of personnel, and also there is a scarcity in equipment to do these actions. It is necessary to increase monitoring activities in wildlife in order to have a more real number regarding rabies positive cases and to show a more complete epidemiological surveillance.

Name: Marcélia Fernandes

PP42

Email: hruthner@yahoo.com.br

Session: Rabies in Domestic Animals

Country: Brazil

Title: Spillover of rabies virus in cats in State of Rio Grande do Sul, southern Brazil

Authors: Marcelia Emanuele Sad Fernandes[1], Adriele Naiara F Gregório[1], Laura Lopes de Almeida[2], Julio Cesar Almeida Rosa[2], Jose Carlos Ferreira[2], Pedro Carnieli Junior[1], Rafael de Novaes Oliveira[1], Juliana Galera Castilho Kawai[1], Paulo Michel Roehe[3], Helena Beatriz de Carvalho Ruthner Batista[1]

Affiliations: [1] Pasteur Institute of São Paulo, São Paulo, Brazil; [2] Veterinary Research Institute Desidério Finamor, Eldorado do sul, Rio Grande do Sul, Brazil; [3] Federal University of Rio Grande do Sul, Institute of Basic Sciences of Health, Porto Alegre, Rio Grande do Sul, Brazil

Abstract: Epidemiology of rabies in Brazil is variable according to the region. In State of Rio Grande do Sul (RS), southern of Brazil urban rabies transmitted by dogs and cats has not been detected since 1988, rabies in cattle transmitted by hematophagous bats *Desmodus rotundus* is endemic and many species of non hematophagous bats were found infected with Rabies virus (RABV) in the State. The aim of this work is to describe three cases of rabies in cats in State of RS. The first case occurred in 2014 in the city of Passa Sete. The second and the third cases were recorded in 2015 in the Cities of Capão do Leão and Rio Grande. All cases were reported to the public health authorities. The public health service collected each animal and sent to the reference laboratory of rabies in RS. Diagnosis of rabies was performed by Direct Immunofluorescence Test (DFAT) and Mouse Intracerebral Test (MIT). In addition the strains were submitted to genetic characterization with RT-PCR, sequencing and phylogenetic analysis of nucleoprotein gene (N) of RABV. By alignment the sequenced fragment of strains with equivalent RABV sequences available at GenBank, the highest identity was found between the first strain and RABV genetic lineage whose natural reservoirs are the vampire bats *D. rotundus*. For strains recorded in 2015 the highest identity was found with RABV genetic lineage whose natural reservoirs are the insectivorous bats *Tadarida Brasiliensis*. Our results highlight for the importance of determination of the species of origin, and indicate that all cases of rabies in cats described were an occasional spillover from bats. The study was essential to appropriate management of sanitary vigilance resources.

Name: Ruth Steinberger

PP43

Email: ruth@spayfirst.org

Session: Rabies in Domestic Animals

Country: United States

Title: Practical applications of calcium chloride dihydrate for non-surgical castration of male dogs

Authors: Ruth Steinberger

Affiliations: Spay FIRST!, Oklahoma City, Oklahoma, USA;

Abstract: Intratesticular injections of calcium chloride dihydrate in ethyl alcohol are a well documented, cost effective method of permanent non-surgical castration of dogs (http://www.actavetscand.com/content/56/1/62). The two ingredients are not patented, making it universally available to veterinarians. Socio economic factors are cited as reasons why people in developing nations keep and/or feed male dogs at a rate far greater than females, with males comprising up to 80 percent of free roaming canine populations (Rowan, Jackson). While altering females is key to reducing the overall number of dogs, intact male dogs form packs while pursuing a female in estrus, resulting in bites and maulings of people and other animals. Our hCG challenged testosterone tests confirmed earlier findings of Jana and Leocci that intratesticular injections of calcium chloride significantly reduce testosterone levels. Using a technique developed by H. W. Haney, DVM (US), testosterone levels were reduced in some cases to the level of a surgically castrated dog. Reducing testosterone is vital to reducing libido in male dogs. In chronic poverty, the demand for canine sterilization/rabies clinics far exceeds the capacity to provide them. The cost of the calcium chloride for a medium sized dog is less than the cost of the rabies vaccine. This process is administered under mild sedation which minimizes recovery time, making the application practical and safe during times of year in which general anesthesia would not typically be administered to outdoor animals. The necessary equipment costs under one hundred dollars and is easy to transport. The use of injectable calcium chloride facilitates a strategy for reaching more animals with less money and in challenging locations. While scientific papers have been published on this compound since 1978, this presentation would focus on the convenience, economy, and models for incorporating injectable calcium chloride at the time of a rabies vaccination clinic.

Name: Iana Suly Santos Katz

PP44

Email: ianasuly@gmail.com

Session: Pathogenesis and Molecular Epidemiology

Country: Brazil

Title: Analysis of the virulence and pathogenesis of street rabies virus strains associated with Eptesicus bats

Authors: Natalia Langenfeld Fuoco[1], Karen Miyuki Asano[1], Luciana Botelho Chaves[1], Orlando Garcia Ribeiro[2], Karin Corrêa Scheffer[1], Iana Suly Santos Katz[1]

Affiliations: [1] Pasteur Institute of São Paulo, São Paulo, Brazil; [2] Butantan Institute, Immunogenetics Laboratory, São Paulo, Brazil

Abstract: The rabies virus (RABV) has been observed in a significant number of species of the *Chiroptera* order. It is known, that some characteristics of these hosts, such as high population density, the intense social interaction and high displacement capability, population viral perpetuation processes and transmission of rabies virus in bats, therefore of critical epidemiological importance. Thus, the same groups have studied to obtain a better understanding of the RABV pathogenesis in bats. In this context, this study aims to analyze virulence and pathogenesis of RABV associated with Eptesicus bats. For this, after replication of the virus in N2A cells, were performed growth kinetics, cell-to-cell spread of RABV samples isolates from *Eptesicus* sp (3041-V/2014 and 3333-V/2014). For evaluation of the pathogenesis, RABV were inoculated by intradermical route in mice and clinical signs observed for 40 days. All parameters were compared with fixed virus (CVS-31). We observed that the 3041-V/2014 strain showed lower replication rate, higher rate of spread and intermediate pathogenicity than the 3333V-/2014 and the CVS strains. However, the 3333-V/2014 strain showed higher replication rate, slowly cell-to-cell spread and lesser pathogenicity in mice when compared to the other samples. The CVS strain was more pathogenic in vivo. Our results indicate that the 3333V-/2014 strain showed lower virulence and pathogenicity compared with the 3041V-/2014. These results, shows that there is a variability of strains maintained in *Eptesicus* bats. It is noteworthy that the samples used in our study are from different regions of the country, where the 3333V-/2014 isolated from bats found in Paraná and the 3041V-/2014 from bat found in the Araraquara region in São Paulo, which may explain the variability observed. These results can contribute to a better understanding of the pathogenesis of the rabies virus maintained in the *Eptesicus* bats.

Name: Tisheena Talk

PP45

Email: Tad.Theimer@nau.edu

Session: Wildlife Rabies

Country: United States

Title: Bird feeders as locations for skunk uptake of oral rabies vaccine baits

Authors: Tisheena Talk[1], Tad Theimer[1], Shylo Johnson[2], Amy Gilbert[2], David Bergman[3]

Affiliations: [1] Northern Arizona University, Flagstaff, Arizona, USA; [2] US Department of Agriculture, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA; [3] US Department of Agriculture, Wildlife Services, Arizona, USA

Abstract: In any oral rabies vaccination effort, maximizing bait uptake increases the chance of achieving herd immunity. Traditionally, oral baits have been deployed haphazardly in urban/suburban areas wherever animals are likely to encounter them (vacant lots, alleyways, culverts). Because striped skunks frequently use seed spilled below bird feeders, we tested whether placebo bait uptake was higher at bird feeders than at sites like those where baits would fall via traditional methods. We obtained access to existing bird feeders by finding volunteers from the community either via door-to-door walking surveys or e-mail contact through the local chapter of the Audubon Society. We placed placebo ONRAB baits under 26 bird feeders and at 26 paired, non-feeder locations in suburban Flagstaff and Sedona, Arizona in June and July 2015 and monitored bait uptake using infrared, motion-sensitive trail cameras over four nights. At feeders, we asked homeowners to place a bait below the feeder each evening, record its fate the next morning and replace the bait if chewed or taken. Survival analysis indicated that placebo baits were chewed or removed by skunks significantly faster from under feeders (Chi-square = 6.9, $P < 0.01$), with the initial bait removed from 14/26 (54%) feeders and from 5/26 (19%) non-feeder sites. In addition, by having homeowners replace baits that were chewed or removed, an additional 11 skunks took placebo baits from under feeders. On a per-bait basis, placing baits at feeders was 5 times more effective than placing baits haphazardly. Recruiting homeowners to place baits under existing bird feeders could effectively supplement traditional bait placement techniques in urban/suburban areas and 1) increase the probability of reaching sufficient vaccination levels to achieve herd immunity, 2) allow more rapid dispersal of baits during outbreaks, and 3) actively engage the public in rabies control efforts.

Name: Fernando Favian Castro Castro

PP46

Email: Fernandomorcegos@gmail.com

Session: Bats and Lyssavirus: Host and Virus Diversity

Country: Brazil

Title: Phylogenetic analyses of the hematophagous bat Desmodus rotundus in Valle del Cauca, Colombia

Authors: Fernando Favian Castro Castro and Jaime Eduardo Flores Muñoz

Affiliations: São Paulo State University, Faculty of Veterinary Medicine and Animal Science, Veterinary Hygiene Department and Public Health, Botucatu, SP, Brazil

Abstract: Phylogenetic analyses of the hematophagous bat *Desmodus rotundus* were performed in villages belonging to Valle del Cauca, Cauca and Amazonas regions. Samples were mainly collected at hematophagous bat shelters located in Victoria, Águila, Obando, Cartago, Zarzal, San Pedro, Cerrito and Palmira villages belonging to Valle del Cauca region, two samples at Mercaredes which belong to Cauca, two samples at Puerto Nariño which is part of Amazonas region. Epithelial tissue samples were collected from bat's patagium, and submitted to PCR using primers 16 SL and 16SH amplifying mitochondrial DNA of 50 vampires, these products were sequencing resulting in eight haplotypes being the most common presented in 43 individuals. It was employed software MEGA5® in the maximum likelihood and maximum parsimony analyses. GenBank sequences of mitochondrial markers s16 rRNA from Brazil, Venezuela and Costa Rica were used for comparison. Higher similarity between vampire bats from Valle del Cauca and Cauca region, located in the southwest Colombia and vampire bats from Costa Rica and Venezuela was found. In the present study the genetic profiles correlated with the phylogeographic aspects of different populations was performed and a connection between different haplotypes of existing clades was found according to the phylogenetic tree and haplotype net, possible being generated during hematophagous bats migration over the mountain-chain and rivers crossing Colombia.

Name: April Davis

PP47

Email: april.davis@health.ny.gov

Session: Bats and Lyssavirus: Host and Virus Diversity

Country: United States

Title: Overwintering of rabies virus in silver haired bats (Lasionycteris noctivagans)

Authors: April D Davis[1], Shannon A Morgan[2], Michelle Dupuis[1], Craig E Pouliott[1], Jodie A Jarvis[1], Robert J Rudd[1]

Affiliations: [1] New York State Department of Health, Wadsworth Center, Rabies Laboratory, Slingerlands, New York, USA; [2] University at Albany SUNY, Department of Biological Sciences, Albany, New York, USA

Abstract: Silver haired bats (*Lasionycteris noctivagans*) are semi-colonial, migratory tree bats that have infrequent contact with humans. Despite the species rarity, the silver haired bat (SHB) rabies variant (RV) is the most commonly reported RV in domestically acquired human rabies cases in the US. Unlike big brown bats (*Eptesicus fuscus*) and little brown bats (*Myotis lucifugus*), SHB are not obligate hibernators. It is unknown if RV can overwinter in hibernating SHB or is only maintained in bats that migrate to warmer climates. To better understand RV overwintering in this species, SHB were inoculated intramuscularly with either a homologous RV (LnV1) or one of two heterologous RV (EfV2 and MIV1). One week following inoculation, SHB were placed in a hibernation chamber for 6 weeks. Our results demonstrate that rabies virus can overwinter in SHB yet the incubation period was extended 6 weeks when compared to bats maintained at ambient temperatures. However, unlike bats maintained at ambient temperatures, viral RNA was found in most tissues examined. Additionally, we found that the longer the incubation period, the greater the viral dissemination. Similar to our previous studies, SHB were most susceptible to a homologous variant.

Symposium on Human Rabies Prevention and Treatment

Abstracts

Name: Charles Rupprecht

Email: charles_rupprecht@yahoo.com

Session: Webinar

Country: United States

Title: A primer on pre- & postexposure rabies prophylaxis

Authors: CE Rupprecht, VMD, MS, PhD, Professor (Adjunct), The Wistar Institute, Philadelphia, PA

Abstract: Human rabies may be prevented by avoiding exposure or application of appropriate prophylaxis after viral exposure. Exposure is defined as any bite, trans-dermal or mucosal contact with infectious material, such as saliva or brain tissue. Preexposure vaccination is provided to those at high risk of exposure, such as veterinary professionals, diagnosticians, animal control staff, wildlife workers, cavers, and certain travelers to areas of high endemicity, in part on the basis of duration of stay, activity and local availability of modern biologics. As long as an individual remains at risk, dependent upon categorical extent, serological surveillance for rabies virus neutralizing antibody should occur routinely, from every 6 months to 2 years. If antibody titers fall below an acceptable level, a single vaccine booster is administered. Regardless of titer, once an exposure occurs, two doses of vaccine, administered on days 0 and 3, provoke a rapid anamnestic response in the previously vaccinated person. For the naïve person, after a thorough risk assessment (encompassing information on the biting animal, its availability, the circumstances of the event, etc.), wound care, infiltration of rabies immune globulin and administration of cell culture vaccines, form the hallmark of postexposure prophylaxis. Survivorship is virtually assured when prophylaxis is both timely and in keeping with public health recommendations. Over the next five years, many realistic expectations from a global perspective in the field are anticipated, including: new purified, sub-unit and recombinant vaccines; the first licensed monoclonal antibodies; more simplified immunization schedules; alternative tests for vaccine potency; and emerging producers and markets, particularly in Asia.

Name: Jennifer House

Email: jennifer.house@state.co.us

Session: Webinar

Country: United States

Title: Public health challenges in rabies prevention and animal management

Authors: Jennifer House, DVM, MPH, State Public Health Veterinarian, Department of Public Health & Environment, Denver, CO

Abstract: Rabies is preventable in both people and pets. Unfortunately there are many challenges in rabies prevention and management that public health officials must overcome. Some of the challenges surrounding pet rabies prevention include lack of compliance with rabies vaccination, inconsistent messaging between agencies, misunderstanding of quarantine regulations, inability to test rabies reservoir species that have exposed pets, and limited locations where rabies reservoir species can be processed and tested. In addition to these pet challenges there are also numerous problems that come up when addressing human risk; some of those challenges include proper risk assessment, access to post exposure prophylaxis, mis-administration of post exposure prophylaxis, and patient compliance with dosing schedule.

Name: Richard Franka

Email: rpf@cdc.gov

Session: Webinar

Country: United States

Title: Plato's Cave: shadows or reality? What are future diagnostic targets for human rabies?

Authors: Richard Franka, DVM, PhD, MPH, Director, OIE Reference Laboratory for Rabies, Centers for Disease Control & Prevention, Atlanta, GA

Abstract: Early laboratory diagnostic of rabies is a critical component of not only patient's prognosis and choices about possible therapeutic interventions but also about decisions regarding risk assessment and prophylaxis of contacts. Since Zinke's demonstration of transmission of rabies through saliva in 1804 and Negri's discovery of eosinophilic inclusion bodies in 1903, rabies laboratory diagnostics have focused on the detection of either rabies virus and its antigen (DFA, MIT), antibodies (RFFIT, IFA, FAVN) and later also on its RNA (PCR) as primary diagnostic targets. However, given unique pathobiology of solely neuronal spread of rabies virus and its ability to initially avoid the immune system, these markers cannot be detected earlier than few days before disease symptoms onset. Two major questions raising from differences between medicine and individual patient care and public health and population preventive care influence current directions in rabies laboratory diagnostics. On one hand, modern rapid high-through output technologies are being developed to detect single viral particle or its nucleic acids. On the other hand, simple, low tech, field suitable laboratory methods with high specificity and sensitivity are critically needed to be implemented in canine rabies-endemic countries in decentralized fashion to promote rapid laboratory based PEP decision-making process. Second, largely neglected issue, is the identification and selection of proper diagnostic targets to confirm rabies infection in the incubation and in early morbidity periods. Since antigen and RNAs, most often used markers, are currently not detectable during incubation period, their detection during morbidity period only confirms clinical diagnosis in a patient presenting with symptomatic encephalitis. What is the future of human rabies laboratory diagnostics? What progress was made in past 40 years? Should we continue to enhance techniques to detect real culprit (viral components) or should we focus more on earlier detectable traces of virus presence (sets of small-molecule metabolites or single nanoparticles i.e. shadows)? Past and current rabies laboratory diagnostic techniques for rabies will be reviewed herein and potential novel approaches, their benefits and disadvantages as well as applications will be discussed in an interactive format.

Name: Rodney Willoughby

Email: rewillou@mcw.edu

Session: Webinar

Country: United States

Title: Care of rabies – time for a randomized clinical trial

Authors: Rodney Willoughby, MD, Professor, Children's Hospital of Wisconsin, Milwaukee, WI

Abstract: Human rabies survivors are increasingly reported. Treatment attempts persist despite expert opinion. Rabies therapy is now actively investigated. A continuum of rabies severity has been proposed and new clinical cognates defined. Laboratory diagnosis remains difficult and geographically restricted. The current definition of rabies has been challenged. Recommended treatment strategies vary from palliation to critical care. Experimental approaches are contradictory, and often not registered or reported. Survival curves can be constructed from reported cases. There are no animal survival models. Various thermal, biological drug, pharmacological, immunotherapeutic and molecular biologic strategies are proposed. There is sufficient equipoise between conventional critical care and experimental treatments to justify randomized clinical trials. Ethical, financial and logistical challenges are formidable but can be solved. The benefits of such trials include convergence of expertise, standardized and centralized data collection, ethical and cultural validation, sequencing and bioinformatics support, and bio-repositories for future research.

Name: Robin Levis

Email: robin.levis@fda.hhs.gov

Session: Webinar

Country: United States

Title: Human rabies vaccines and alternate potency assays

Authors: Robin Levis, PhD, Deputy Director, Office of Vaccines Research and Review, Food & Drug Administration, Silver Spring, MD

Abstract: Human rabies vaccine potency is currently measured using the NIH potency test, a test which measures protection against rabies virus challenge in immunized mice. While the test is well accepted as a measure of vaccine potency/efficacy, it has been recognized for decades that the assay has several critical problems. These include; the expense and length of the test, the use of a large number of animals, a lethal challenge step, and a high degree of variability between assays. Vaccine manufacturers and regulatory authorities for both veterinary and human vaccines have been working for some time to develop an alternative assay to measure vaccine potency. Despite significant discussion and the development and testing of several alternative assays it has been difficult to move forward with the licensure of an alternative potency assay. This presentation will introduce considerations for an alternative potency assay based on an ELISA assay to measure the rabies G glycoprotein and provide a report of an ongoing pre-collaborative study and future strategies as discussed at a recent workshop of the EPAA rabies working group.

Name: Zhen Fu

Email: zhenfu@uga.edu

Session: Webinar

Country: United States

Title: The importance of virus neutralizing antibodies (VNA) in clearing rabies virus from the CNS: can VNA be used for clinical therapy?

Authors: Zhen F. Fu, DVM, PhD, Professor, Veterinary Virologist, University of Georgia, Athens, GA

Abstract: One of the major hallmarks for rabies is the almost 100% mortality. There is still no proven therapy for clinical rabies despite of the development of Milwaukee protocol. It has been known for many years that most of the rabies patients (>70%) do not develop virus neutralizing antibodies (VNA) at the time of death, which has also been observed in laboratory animals including mice, dogs and skunks after experimental infection with wt rabies virus (RABV). It has been demonstrated that wt RABV is incapable of inducing innate (inflammation, DC activation) and adaptive immunity (VNA), most likely due to the restricted expression of the glycoprotein (G). Thus evasion of the host immunity is one of the important pathogenic mechanisms for rabies. It has been further demonstrated that VNA alone in the periphery is unable to clearing an established RABV infection in the CNS unless present in the CNS. It has been demonstrated that wt RABV in the CNS can be cleared and infected animals saved by direct intracerebral administration of attenuated or recombinant RABV, which not only lead to the production of VNA in the periphery, but also enhance the Blood-brain Barrier (BBB) permeability. It has been further shown in mice that intravenous administration of VNA in combination with chemokines that enhances the BBB permeability can clear wt RABV from the CNS and prevent the development of rabies. With all these new discoveries, is it possible to try such modalities alone or in combination in large animals or even in humans?