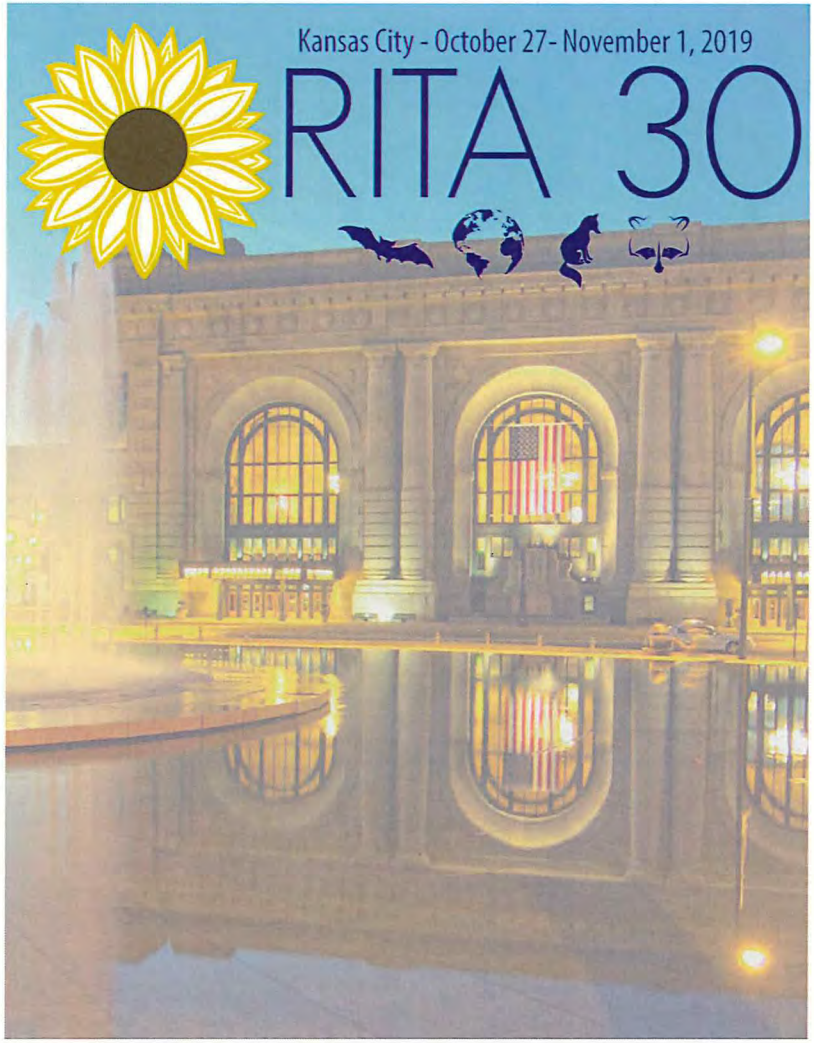

Welcome!

On behalf of the RITA 30 Organizing Committee, I welcome you to the 30th International Rabies in the Americas (RITA) conference.

Kansas State University (KSU) College of Veterinary Medicine is privileged to host this event, held for the first time in Kansas City, the Heart of America. The surrounding area spanning from Kansas State University in Manhattan, Kansas to the University of Missouri in Columbia, is known as the KC Animal Health Corridor; the single largest concentration of animal health and nutrition companies in the world. This year's RITA focuses on the Zero by 30 effort to end human deaths from dog-mediated rabies. As primary organizers of the meeting and through our work, the KSU Rabies Laboratory is honored to be part of this effort.

On this 30th anniversary of RITA, we highlight RITA's history of providing an inviting environment to foster opportunities for researchers, human and animal health professionals, wildlife biologists, international, national and local rabies program managers, laboratory workers and students engaged in rabies prevention and control efforts. Many collaborations have been initiated, discussions shared, and successes celebrated during past RITA conferences and events. We look forward to advancing the knowledge and applications of lessons learned to meet the challenges of the Zero by 30 mission during this conference.

The organizers would like to thank all the presenters, both oral and poster, for sharing their work at RITA 30 and especially our invited speakers. To the International Steering Committee and past organizers, we extend our sincere thanks for invaluable guidance. We acknowledge and thank our generous sponsors for helping make this event a success.

It is our hope that you will find yourself challenged to consider problems in different ways, that you return to your workplaces/schools with genuine benefit, and that you find yourself part of the global effort to eliminate human rabies deaths. Enjoy your time in the Heart of America and explore the history, food and attractions of Kansas City, both the Kansas and Missouri sides!

Best wishes for a pleasant and educational meeting,

Susan M. Moore
Chair, RITA 30 Organizing Committee
Rabies Laboratory Director, Kansas State University

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Rodney Rohde – Texas State University – Associate Dean for Research - USA
Rodney Willoughby – Physician – Children's Hospital of Wisconsin - USA
Rolan Davis - Kansas State University – KSVDL Rabies Laboratory - USA
Ryan Wallace – Centers for Disease Control - USA

ORAL PRESENTATIONS

Pathogenesis/Immunology

Moderators: Thomas Mueller and Rodney Willoughby

Inhibition by Rabies Glycoprotein Neurotoxin Like Peptide Differs Between Nicotinic Acetylcholine Receptor Subtypes

Karsten Hueffer, Shelly Thao, Chanta Spain, Maegan Weltzin

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Animals infected with rabies show changes in locomotor, breathing, and aggression behaviors. These behaviors are controlled by brain regions that express a variety of nicotinic acetylcholine receptors (nAChRs) subtypes. The rabies virus glycoprotein (RVG) interacts with nAChRs, but detailed analysis on these interactions have not been performed. The purpose of this study is to determine the activity and selectivity of RVG on nAChR subtypes, and the associated isoforms, that are involved in the modified behaviors of infected animals. We used a RVG-derived peptide of the putative region that is predicted to interact with nAChRs. Each isoform of the tested nAChR subtypes was expressed in Xenopus laevis oocytes using biased cRNA subunit injection ratios. Two-electrode voltage clamp electrophysiology was used to collect concentration-response data to measure the potency of RVG peptide inhibition on acetylcholine activated nAChRs. Results show that there are differences in RVG peptide inhibition potencies between each nAChR subtype tested. Interestingly, this study shows that the RVG peptide has higher inhibition potency for the α7 nAChR subtype compared to the other examined subtypes. Results identify new molecular targets (nAChR subtypes and the associated isoforms) for the RVG, and expands our understanding of basic mechanisms in molecular rabies host interactions. Future studies will identify specific RVG peptide and nAChR subtype interactions that could lead to enhanced understanding of rabies pathogenesis and aid in the development of new pharmacological approaches to treat rabies infection.

Improving rabies animal models for medical countermeasures through luminescent lyssaviruses

David E. Lowe, Nadia Gallardo-Romero, James A. Ellison, Felix Jackson, Chantal Kling, Laurie Seigler, Christoph Wirblich, Matthias Schnell, and Christina L. Hutson

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Background: Developing a rabies treatment is complicated because infection with Rabies lyssavirus (RABV) has a long prodromal stage and early signs of rabies in animal models can be non-specific. This requires lengthy studies with large numbers of animals. Our objective was to develop an animal model whereby RABV dissemination can be detected in living animals prior to clinical signs by using a light-emitting virus.

Methods: In this study, we evaluated two luminescent mouse models of experimental rabies. One model used a RABV-N2c with the nanoluciferase gene inserted in the viral genome (RABV-NL). The other model used a RABV-N2c with the Cre gene inserted in the viral genome (RABV-Cre) and a complimentary transgenic mouse. Mice were infected intramuscularly with RABV-NL (n=10), RABV-Cre (n=10), or unaltered RABV-N2c (n=10) and observed for 21 days.

Results: Luminescence was detected in the central nervous system for both models ~24 hours before specific rabies signs appeared. However, light was not detected in peripheral nerves upon post-mortem examination. All mice with CNS luminescence were confirmed rabid by molecular diagnosis. Rarely, luminescence was detected in other tissues, such as the eyes and briefly at the injection site. High mortality was observed in mice infected with RABV-NL and the unmodified RABV-N2c. However, mortality in the RABV-Cre-infected animals was reduced by half.

Conclusion: Luminescent rabies models can detect CNS infection before the display of clinical features. These models allow us to reduce and refine the use of laboratory animals in the development and testing of new MCMs for rabies encephalitis.

Favipiravir treatment of human rabies

Rodney E. Willoughby, MD and Carolyn R. Epstein, MD. Medical College of Wisconsin, Milwaukee, Wisconsin, USA; BioLogics Services, Inc., Waltham, Massachusetts, USA, and Fujifilm Pharmaceuticals USA, Valhalla, New York, USA

Medical College of Wisconsin

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Background: Survival from human rabies in increasing worldwide through use of critical care without effective antiviral compounds. Favipiravir is an orally administered antiviral with a broad spectrum of activity against RNA viruses including influenza A, ebolavirus, and rabies virus. Efficacy of favipiravir prophylaxis against rabies in animal models depended on dose. Favipiravir was effective in other animal encephalitis models. Favipiravir dosing for treatment of ebola in children and adults has been recommended, but bioavailability in the human central nervous system (CNS) is unknown.

Methods: Favipiravir was provided by Fujifilm USA under emergent investigational new drug authorization by the US Food and Drug Administration to 6 patients with confirmed rabies (dog n=2; vampire bat n=2; insectivorous bat n=2) in 3 countries. All patients were treated with the Milwaukee protocol.

Results: Three adults and three children received favipiravir for treatment of human rabies. There were logistical challenges to providing investigational drug in different countries. Survival was 16% including one patient who had therapy discontinued by family request. There was no effect on salivary viral load. In patients who received favipiravir in the first week, peripheral autonomic function was preserved when compared to other patients treated with the Milwaukee Protocol. Pharmacokinetic studies of bioavailability in CSF and plasma are planned.

Conclusions: Favipiravir showed possible clinical effect when administered early during rabies treatment, but there was no survival benefit at current dosing. Analysis of favipiravir bioavailability in the human CNS (and the need for dosage adjustment) is planned.

PRE-EXPOSURE PROPHYLAXIS FOR RABIES MEDIATED BY VAMPIRE BAT IN HUMAN POPULATION LIVING AT RISK IN AMAZONIA REGION, STATE OF PARÁ, BRAZIL

ROCHA Felipe1, VARGAS Alexander2, ZANETTE Larissa Cacho1, VIANNA Raphael Schneider1, LIMA Daniel Magalhães1, VAZQUEZ Manuel Jose Sanchez1, MOLINA-FLORES Baldomero1, DUARTE Paulo de Camargo1, VIGILATO Marco Antonio Natal1, ROCHA Silene Manrique2, BEGOT Alberto Lopes3, ESTEVES Fernando Augusto Lourenço3, ANDRADE Jorge Alberto Azevedo3, COSTA Wagner Augusto4, WADA Marcelo Yoshito2, POMPEI Júlio Cesar Augusto1, COSIVI Ottorino1.

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In 2018, the last outbreak of human rabies mediated by hematophagous bats occurred in Melgaço, State of Pará, in a region inside of the Amazon Basin, where 11 cases of human rabies were reported by Ministry of Health of Brazil - 10 in children (3 to 15 years old) and 01 in adult. An increase in the rabies incidence has been observed on populations living in this ecosystem due to human expansion over sylvatic environments and to the difficulty of access to health services. Pre-exposure prophylaxis vaccination of human population in risk areas for hematophagous bat-mediated rabies was not incorporated as a routine public health action in Brazil - only as a preventive action in population to block the expansion of an outbreak. Therefore, during the period from September 9th to 29th of 2019, the Ministry of Health of Brazil and the Pará State Secretary of Health, with the support of PANAFTOSA-PAHO/WHO and Institute Pasteur, will promote a pilot action for human rabies vaccination to evaluate the action as a public health measure. The pilot will take place in an area of 560km2 of the Pacajá River, Portel municipality, State of Pará. By the use of fluvial transportation, the action aim to reach an estimated population of 2.500 persons, that will receive a 02 doses pre-exposure prophylaxis protocol. Logistic and economic analysis and the characterization of the pilot population will generate results to subsidy future actions for neglected population in all Amazonia Region.

ORAL PRESENTATIONS

Bat Rabies

Moderators: April Davis and Luis Lecuona

The Potential for Urban Water Sources to Increase Rabies Transmission between Bats and Mesocarnivores

[1]Lias Hastings, [1]Carol Chambers, [1]Tad Theimer, [2]David Bergman

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From 2001 to 2009, Flagstaff, Arizona, experienced three separate rabies outbreaks in populations of striped skunks and grey foxes. Each outbreak involved a rabies variant that is typically found in big brown bats. We hypothesized that in the arid southwestern United States, where free water is often limited, artificial, urban water sources could act as centers for rabies transmission from bats to mesocarnivores. We tested the ecological potential for this hypothesis by using ultrasonic acoustic recorders to assess bat activity and camera traps to estimate mesocarnivore abundance. We compared paired water versus non-water locations twice during the summer, once before the summer monsoon season and once during the monsoon season when surface waters would be more available. Calls of all bats combined, and that subset of calls with acoustic characteristics consistent with big brown bats, were recorded significantly more at watered sites in both study periods, indicating greater use of watered sites regardless of the availability of water due to monsoonal activity. In both sampling periods, raccoons were photographed significantly more often at watered than non-water sites, but striped skunks and grey foxes were not. These preliminary data suggest that bats are more abundant near water sources, therefore there is a greater potential for transmission of rabies near urban water sources because bats are more abundant. Dense housing around many urban water sources make this a concern for public health.

The feasibility of controlling rabies in bats through topical application of vaccine.

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Rabies transmitted by vampire bats (Desmodus rotundus) in Central and South America is a significant threat to humans and also a tremendous economic burden due to widespread cattle mortality and prophylaxis. Other bat species (e.g. Eptesicus fuscus and Tadarida brasiliensis) are major reservoirs of rabies for humans, domestic animals and wildlife in North America. We recently developed a new recombinant rabies vaccine specifically for bats using an in silico antigen designer tool to create a mosaic glycoprotein (MoG) gene with available sequences from the rabies Phylogroup I glycoprotein. This sequence was cloned into raccoonpox virus (RCN) and the efficacy of this novel RCN-MoG vaccine was tested in big brown bats (E. fuscus). Bats immunized with RCN-MoG by the oronasal route (100%) or treated topically with the vaccine in glycerin jelly (83%) survived rabies challenge at a much higher rate than control bats (11%). Ongoing work includes development of improved vaccine constructs and methods to topically vaccinate bats en masse, additional vaccine efficacy and rabies challenge studies in vampire bats and big brown bats, and pilot field studies to test application methods using biomarkers in lieu of vaccine. If successful, topical application of vaccine could be an effective method of vaccinating large numbers of bats, providing another tool for managing rabies.

Vampire...Pigs? Assessing the impact of feral swine on the northward advancement of vampire bats towards the United States.

[1]Michael Houts, [2]David Bergman

[1]Kansas Biological Survey, University of Kansas, Presenter

[2]USDA Wildlife Services, Phoenix AZ.

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Wildlife vectors for rabies in the Unites States include raccoons, skunks, gray fox, and insectivorous bats. The transmission of rabies from these vectors to domestic animals, livestock, and humans is relatively rare. In South America, Central America, and Mexico, however, the vampire bat is a rabies vector that has an economic impact to livestock production and at times affects human health. In recent years, the vampire bat population has been slowly approaching the United States from Mexico. Climate change and habitat loss have been indicated as possibly facilitating this expansion, but its expansion may also have been slowed due to the lack of readily available food sources (blood). The risk of rabies impacting livestock and people in the Unites States greatly increases if the vampire bats establish a population that has access to the abundance of feral swine, cattle, goat, and sheep populations of southern Texas.

Using a uniform mapping grid that spans Mexico and the United States, researchers are attempting to analyze vampire bat range, feral swine density, and livestock density in conjunction with environmental variables. The multiscale Nested Hexagon Framework developed at the University of Kansas will be used to map what areas could provide the preferred combination of resources to allow vampire bats to establish a population in the United States. With this data, researchers hope to identify what areas could be at the highest risk for vampire bat/livestock interaction and where the bats might go if the range continues to expand.

Challenges and avenues in bringing the novel Kotalahti bat lyssavirus (KBLV) to live and characterize its properties

Conrad M. Freuling, Niina Tammiranta, Tiina Nokireki, Elisa Eggerbauer, Luca Zaeck, Madlin Potratz, Claudia Wylezich, Sten Calvelage, Dirk Höper, Thomas Müller, Stefan Finke

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There is a growing diversity of bat-associated lyssaviruses in the Old World. In August 2017, a dead Brandt's bat (Myotis brandtii) found in Eastern Finland in the municipality of Leppävirta, in the village of Kotalahti, tested positive in the fluorescent antibody test (FAT). Phylogenetic analysis of N-gene sequence revealed that the virus differed from other known lyssaviruses, with highest nucleotide identities with KHUV, ARAV, BBLV, and EBLV-2. The virus was designated as Kotalahti bat lyssavirus (KBLV), a tentative novel member of the genus Lyssavirus. Because the bat was in an autolyzed state, isolation of KBLV was neither successful after four consecutive cell passages on MNA cells nor in 3-week old and suckling mice. Next generation sequencing (NGS) was applied using Ion Torrent ™ S5 technology coupled with target enrichment via hybridization-based capture (myBaits®) which resulted in the complete genome. While for unknown reasons, the rescue of the synthetic KBLV clone was not successful, trans-complementation of SAD-L16 G-deficient infected cells by plasmid-expressed KBLV-G resulted in viral particles, albeit with a low titer. Using the virus from the supernatant as test virus, sera from human vaccinees demonstrated a similar level of cross-neutralization compared to CVS as EBLV-2 and ABLV, confirming the phylogenetic grouping into phylogroup I and suggesting that RABV vaccines also confer protection against KBLV.

There is ongoing efforts to establish a BBLV backbone for eventual rescue of chimeric viruses which would allow further characterization of viral properties including antigenic typing and pathogenicity studies.

Outbreak of bat-transmitted human rabies, Marajó island, Brazilian Amazon, 2018.

Jorge Alberto Azevedo Andrade; José Maria dos Santos Coelho Júnior; Neuder Wesley França da Silva; Martha Elizabeth Brasil da Nóbrega; Alberto Lopes Begot; Fernando Augusto Lourenço Esteves;Elke Maria Nogueira de Abreu; Alexandre Vargas

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Introduction: In 2018, an outbreak of bat-transmitted human rabies caused deaths in riverine dwellers in Melgaço city, Pará State, Brazilian Amazon. Objective: to describe the clinical and epidemiological profile of the patients outbreak of human rabies in Melgaço city, Marajó island, Brazil. Methods: A descriptive and quantitative study was conducted based on secondary data from the Notification Disease Information System - SINAN and outbreak investigation reports. The variables were tabulated using the Excel® 2010 program and simple statistical analysis was performed. Results: 10 deaths have been confirmed human rabies in riverine dwellers in rural area of Melgaço city. All of them were bitten by Desmodus rotundus. Most of them occurred in males (60%), ranging in age from 1 to 9 years (70%); confirmed by laboratory criteria (60%) and by epidemiological and clinical bond (40%); The bites were usually single (80%) shallow type (80%); The signs/symptoms were fever (90%), paralysis (90%), psychomotor agitation (80%), dysphagia (70%), paresthesia (30%), aggression (20%) and Hydrophobia (10%); There was hospitalization in 90% of cases. All of them evolved to death. Conclusion: The investigation revealed that the riverine dwellers had history of attacks before the outbreak, they sleep in homes without walls and/or mosquito nets and they did not know that bats transmit the disease. To control the outbreak, it was performed pre and post-exposure human vaccine schemes, animal vaccination, capture and control of D.rotundus with Warfarin and the distribution of mosquito nets. There is need to improve control measures of epidemiological surveillance.

Increased Detection of Bat Lyssaviruses and Human Imported Case of Rabies in Great Britain in 2018

Hooman Goharriz,Trudy Johnson, David Selden, Nikki Maclaren, Daniel Dorey-Robinson, Shweta Shukla, Daisy Jennings, Leigh Thorne, Denise Marston, Lorraine McElhinney.

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Passive surveillance of bats in the United Kingdom started in 1987. Prior to 2018,, only 15 European bat lyssavirus 2 (EBLV-2) cases had been reported, all from Daubenton's bats, with a rate of no more than 2 cases per year. However, during June-July 2018, an unusual mortality event, coinciding with a prolonged heatwave, was observed in a Daubenton's bat roost. Twelve bats from the known positive colony were submitted for lyssavirus screening. Many of the carcasses were severely decomposed and untestable by fluorescent antibody test (FAT) and virus isolation techniques. An initial FAT positive result in a testable bat led to a retrospective and more detailed investigation of the decomposed bats using extracted RNA from other tissues on real time RT-PCR. We subsequently detected EBLV-2 RNA in four additional Daubenton's bats from the roost (a total of five cases in this single roost in 2018). Three further unconnected EBLV-2 cases were detected in 2018 in Cambridgeshire (England), Northumberland (England) and West Lothian (Scotland). European bat 1 lyssavirus (EBLV-1) was detected in two Serotine bats for the first time in the UK in 2018. A subsequent case of EBLV-1 was detected from the same region in 2019. The acceptance of PCR by the OIE is likely to lead to increased detection of bat lyssaviruses in sub-optimal samples. Public health guidance for UK health professionals and those bitten by a bat has been updated.

In November 2018 we confirmed rabies in a man bitten by a cat whilst on holiday in Morocco. Rapid screening of ante-mortem samples by molecular methods confirmed the presence of Rabies Virus RNA and avoided the need for a post mortem. The findings of both the bat and human testing will be presented.

ORAL PRESENTATIONS

Rabies in Wildlife

Moderators: Amy Gilbert and Richard Chapman

30th Rabies in the Americas – Kansas City, Missouri, United States of America – October 27 – October 31, 2019

Re-emergence of wildlife rabies in golden jackals (Canis aureus), Israel (2017-2018)

[1]Boris Yakobson, [2]Ron King, [1]Marina Ingor, [3]Michal Perry Markovich, and [3]Tamir Goshen.

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Since 1956, red foxes (Vulpes vulpes) and to a lesser extent, golden jackals (Canis aureus) were the primary rabies reservoirs in Israel. During the mid-1970's, a major transition from urban dog rabies variant to sylvatic fox rabies variant occurred. Since 1998, wildlife rabies has been controlled using oral rabies vaccines (ORV). The fox-targeted ORV program using RABORAL V-RG® (V-RG) vaccine led to a dramatic decrease in the number of confirmed rabies cases. From 2012 to 2016, rabies cases numbered 30 or less annually, were located near borders and attributed to intrusion of infected animals from neighboring countries. In a period of 6 month (October 2017 -March 2018), 68 of 93 (73%) reported rabies cases were jackals from an area of about 500 km2 in the Jezreel Valley and its proximity. The majority of cases were in young jackals not affected by the ORV distribution applied in the spring 2017. The disease became established and the virus circulated in jackal populations of the Valley. Massive ORV distribution (up to 150 baits per sq.km) was implemented beginning in October 2017. The outbreak practically ceased by March 2018 with the peak of positive cases occurring in January (19 positive jackals). Thermostability of the V-RG vaccine and manual dispersal of doses after sunset (average daily temperatures in the area during August –October are 350C in the shadow) contributed to stopping the outbreak. Targeting young jackals born between ORV programs and intensive bait densities controlled this event. Such methods will contribute to preventing future outbreaks.

When All Else Fails: Contingency Actions for Raccoon Rabies Control.

[1]Richard Chipman†, [1]Kathy Nelson, [1]Jordona Kirby, [2]Amy T. Gilbert, [3]Charles Rupprecht, [1]Dennis Slate

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Contingency actions (CA's) are an integral part of wildlife rabies control in North America. Since 1999, at least 15 significant CA's have been implemented in the Eastern U.S. as part of raccoon rabies virus variant (RRV) management with oral rabies vaccination (ORV). Typically when a rabies case is documented beyond an existing ORV zone or in a strategically important rabies management area where no bait zone exists, USDA Wildlife Services (WS) conducts a risk assessment and implements a CA. A CA includes enhanced rabies surveillance (ERS) near the index case and may involve trapping, euthanizing and testing raccoons and skunks for RRV. The direct rapid immunohistochemical test (DRIT) developed by the CDC allows WS biologists to perform rabies diagnostics in real-time to facilitate an "early detection-early response" management approach often testing hundreds of samples during CA's. Management options include focal trap-vaccinate-release efforts, spring and fall emergency ORV baiting operations and pre- and post-bait monitoring to document seroprevalence as an index to vaccine induced population immunity. Most recently CA's have been conducted in Alabama (2014-2016), Stark County Ohio (2017-present), Virginia (2017-present), and Tuscararwas County Ohio as a result of a breaches in ORV zones with RRV cases documented west of long standing ORV zones. A timely response, twice a year, high density baiting (150-300 baits/km2) with RABORAL V-RG® (AL/VA) and ONRAB (OH) coupled with intensified ERS and project monitoring has successfully controlled the further spread of RRV in the U.S. thereby avoiding additional human/animal health and economic impacts of RRV.

Evaluation of a commercial blocking ELISA for the detection of rabies virus antibodies in North American wildlife sera

[1]Amy Gilbert, [2]Todd Smith, [1]Heather Sullivan, [2]L. Claire Godino, [3]Charles Rupprecht, [2]P.S. Satheshkumar, [4]and Susan Moore

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Testing for RV antibodies (RVA) is a key diagnostic activity, widely recognized as a measure of resistance to lethal infection. Demonstration of RVA is important for studies addressing individual level immune responses in experimental pathogenesis and vaccination studies, as well as population level immunity due to natural RV exposures or vaccination activities targeting reservoir hosts for disease control and prevention. While neutralization assays have historically been considered the gold standard for measurement of RVA, ELISA-based assays are gaining in popularity and may allow improved standardization across laboratories. We evaluated the sensitivity and specificity of a commercial blocking ELISA (BioPro® ELISA kit, O.K. Servis, Prague, Czech Republic) for detection of RVA in comparison to a neutralization assay, using 1,131 sera from studies involving five wild carnivore reservoirs in North America. Specificity of RVA detection was greater than 90% across all studies in comparison to neutralization data. Sensitivity was greater than 90% in a captive vaccination study with striped skunks (Mephitis mephitis) and active surveillance for natural RV exposure in small Indian mongoose (Herpestes auropunctatus), but lower than 45% in a captive vaccination study with raccoons (Procyon lotor), and monitoring of oral rabies vaccination activities targeting raccoons and coyotes (Canis latrans). Overall, the commercial ELISA kit exhibited greater utility in the demonstration of RVA in captive experimental studies and natural RV exposures. Additional kit optimization is necessary for use with certain wild carnivore reservoirs and for monitoring oral rabies vaccination programs in North America.

Improved Strategies for Distribution of Oral Rabies Vaccines to Manage Raccoon Rabies in the Urban Landscape of Greater Pittsburgh, Pennsylvania, USA.

[1]Timothy J. Linder†, [2]Lori I. Horowitz, [3]Robert L. Hale, [3]Bradley N. Hicks, [3]Betsy S. Haley, [3]Jordona D. Kirby and [3]Richard B. Chipman

[1]United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Pennsylvania Program, Bolivar, PA, USA

[2]Allegheny County Health Department. Pittsburgh, PA, USA

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The USDA, Wildlife Services (WS) cooperatively distributes >9 million doses of oral rabies vaccine (ORV) in the U.S. annually to protect human and animal health and reduce costs associated with rabies in terrestrial carnivores. Increasingly there is a need for innovative tools and strategies to improve logistics, project monitoring and overall effectiveness of ORV programs in urban landscapes. Hand distribution of baits by vehicle is the preferred way to implement ORV programs in urban habitats. The Greater Pittsburgh area in Pennsylvania includes all of Allegheny County and parts of Beaver and Washington Counties, which has been baited with RABORAL V-RG® since 2002. Despite annual hand bait distribution of more than 300,000 vaccine baits, the area consistently has low rVNA seroconversion rates averaging 20.9% and persistent rabies cases. In August 2018, a revised hand baiting strategy was developed by WS and cooperatively implemented in Greater Pittsburgh by WS and the Allegheny County Health Department (ACHD). The revised strategy included standardized 9-km2 cells to facilitate even distribution of baits at the required bait density of 150/km2. Point of Interest (POI) devices were used by each baiting team to geospatially identify the location and number of baits distributed within each cell. During the 2018 campaign, 120 WS employees and volunteers distributed 301,680 baits by hand in 655 hours while driving >6,580 miles. Evaluation of the geospatial information collected identified spatial gaps in vaccine distribution on the landscape, which were addressed using adaptive management for improved bait distribution coverage during 2019.

ORAL PRESENTATIONS

Epidemiology and Surveillance

Moderators: Lorraine McElhinney and Veronica Gutierrez

Rabies Molecular Epidemiology - how, when and why

Susan Nadin-Davis

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The tools of molecular epidemiology have been applied to the study of rabies cases since the early 1990s. Since that time technological advances in nucleic acid characterization and the design of software programs for analysis of such data have revolutionised our ability to characterise the viruses responsible for this disease. Discrimination of viruses circulating in different host reservoirs can be undertaken using partial gene sequences and for routine viral typing this level of analysis is often sufficient. However, datasets comprising longer sequences, particularly of whole viral genomes, can further resolve the viruses associated with a specific host thereby illustrating how the virus evolves over time and revealing landscape features and topography that impact viral spread. Combining genetic studies on the virus and the host can reveal previously unknown virus-host associations while knowledge of host sub-population structure may help explain patterns of disease transmission. Such information can help inform programs seeking to control animal rabies and thus positively impact efforts to minimise the public health consequences of this disease. This presentation will describe the various methods used for rabies molecular epidemiology, indicate what is appropriate for different scenarios and provide examples of how these tools have been used to further our knowledge of how this disease persists.

Rabies Surveillance in the United States during 2018

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Human and animal rabies have been nationally notifiable conditions in the United States since 1944. National rabies surveillance is a laboratory-based system that comprises > 130 state public health, agriculture, and university laboratories performing the standard direct fluorescent antibody test. In addition, the USDA Wildlife Services submitted active surveillance data on animals tested with the direct rapid immunohistochemical test, accounting for about 6% of all animals submitted for rabies testing. Data submitted by 50 states, District of Columbia, New York City, Puerto Rico and the USDA Wildlife Services were analyzed to provide critical information on the temporal, geographic, and demographic occurrence of animal rabies to facilitate its prevention and control. National rabies management decisions, vaccination recommendations, public education, and numerus other rabies activities rely on an accurate portrayal of the national rabies landscape. The present report provides information on the epidemiology of rabies and rabies-associated events in the United States during 2018. Reported cases of rabies by location will be provided with distribution figures for bats, raccoons, skunks, foxes, dogs, and cats. Rabies virus variants identified in domestic and wild animals will also be described with detailed information. The 2018 rabies surveillance report will provide a summary of human rabies cases from January 2003 through September 2019.

Revising the United States rabies surveillance system through electronic laboratory reporting

Julie Cleaton, Jesse Blanton, Xiaoyue Ma, David Lowe, Ryan Wallace, Brett Petersen, Vicki Olson

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Animal rabies is enzootic throughout the United States and is a nationally notifiable condition. Approximately 100,000 animals are submitted for laboratory diagnosis each year, with around 5% testing positive. A network of over 130 laboratories and USDA field biologists generate the diagnostic and epidemiologic data that informs the national rabies surveillance system. Data are transmitted to the national program through multiple methods, including Microsoft Excel files, PDF files, and PHINMS. Multiple non-standardized formats for data sharing require a manual review and cleaning process; delays of up to 18 months in assessing inter-state rabies trends are not uncommon. Reliable and timely methods for data exchange are critical for the surveillance of animal rabies, and have been established at the national level for numerous notifiable diseases. The Centers for Disease Control and Prevention Poxvirus and Rabies Branch has collaborated with the Association of Public Health Laboratories and state public health, agriculture, and academic laboratories to develop a national electronic laboratory reporting (ELR) system for animal rabies. The goal of this collaboration is to accelerate the adoption of standardized Health-Level Seven (HL7) ELR 2.5.1 results to improve national notification mechanisms for animal rabies. So far four labs are sending HL7 messages, with eight others in progress. Based on annual line lists submitted by the states, the HL7 data has had high concordance coming directly from the labs with over 94% matching. The transition to electronic laboratory reporting will allow for improved canine rabies surveillance and faster detection of and response to epizootics.

A One Health approach to rabies management in Manitoba, Canada

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A One Health approach was developed in the province of Manitoba in 2014 to manage human and domestic animal exposures to rabies. Manitoba Rabies Central is a collaboration of 3 provincial departments responsible for animal, human, and environmental health. Since the inception of the program 537 samples from animals suspected of rabies and causing an exposure to a human or domestic animal have been evaluated with 11.3% testing positive, 85.7% testing negative, and 3.0% being unfit for testing. Most of the positive rabies test results came from skunks (52.0%), which accounted for 12.5% of submissions. Dogs and cats accounted for 52.5% of submissions; however, only 18.9% of these animals tested positive for rabies. Domestic animals were more likely to be exposed to a rabid animal (most commonly skunks) than were humans. Humans were more likely to be exposed to dogs and cats (regardless of rabies test result).

ORAL PRESENTATIONS

Canine Rabies Elimination

Moderators: Laura Robinson and Marco Antonio Natal Vigilato

30th Rabies in the Americas – Kansas City, Missouri, United States of America – October 27 – October 31, 2019

From apartment pets to cave dogs: the gradient of free-ranging behavior in a rabies-affected city.

Authors

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In the city of Arequipa, Peru, the reemergence of the rabies virus to the dog population was detected in 2015. Since then, the Ministry of Health has conducted mass dog vaccination campaigns (MDVC) to halt transmission and eliminate rabies from the city. However, the MDVC in Peru only targets owned dogs and ignores important subpopulations: community dogs, stray dogs, and feral dogs.

Cultural values and practices determine the level of supervision and resources dogs receive. The human-made and natural environment may also help to shape how prevalent different free-ranging patterns are. In Arequipa, the process of urbanization occurs from the center to the periphery of the city. We observe more owned free-ranging dogs closer to the periphery and associated, predominately, with social determinants. Recently, in the interface between the peripheral communities and the barren countryside that surrounds the city we discovered a new dog subpopulation: 'cave dogs'. These unowned animals are using caves, some naturally formed, but many which they excavate themselves, to rest, hide, and breed. We conducted multi-year door-to-door surveys to study owned free-ranging dogs and longitudinal ecological transects to estimate the abundance of unowned cave dogs. In these dog subpopulations, we estimated demographic parameters, spatial distribution, hunting habits, and human acquisition of puppies from caves. We discuss our findings and current interventions to control these cave dog populations, and their implications for rabies control. We also explore alternative and innovative control strategies for rabies elimination in this complex dog population.

Rabies vaccine at six weeks of age increases all-cause mortality rate in female but not male puppies: results of a randomized controlled trial in a high-mortality dog population

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It has been proposed that non-live vaccines have non-specific effects that increase all-cause mortality in females but not males in high-mortality populations. We investigated this proposition in a randomized controlled trial in a population of owned, free-roaming dogs. Our objectives were to evaluate the effect on survival in puppies of an injection of rabies vaccine (RV)—a non-live vaccine—against injection of sterile water (SW), and to determine whether this effect was modified by sex. The study design was a single-site, owner-blinded, randomized, placebo-controlled trial in South Africa. At six weeks of age, puppies within litters were randomly assigned to receive a subcutaneous injection of either RV or SW. The primary outcome was death due to any cause through seven weeks after injection. Effect size was estimated using a mixed-effects Cox model stratified by body weight and adjusting for sex, with a random effect for litter. 358 puppies were randomized (179 per group). Mortality rate was 2,664/1,000 dog-years in the RV group and 1,955/1,000 dog-years in the SW group (hazard ratio 1.35; 95% confidence intervals 0.83–2.18). The effect of RV on survival was modified by sex (p-value 0.02), with the hazard ratio in females 3.09 (95% CI 1.24–7.69) and the hazard ratio in males 0.79 (95% CI 0.41–1.53). Rabies vaccine at six weeks of age increased all-cause mortality in female puppies, with no evidence of an effect in male puppies. Further randomized controlled trials should be conducted in other high-mortality dog populations to confirm these findings.

The epidemiology of canine rabies in Santa Cruz, Bolivia (2017-2019)

Fidel Villegas Anze

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During 2017, canine rabies was reported officially and confirmed in seven of the nine Bolivian departments1. Despite periodic dog vaccination campaigns in the major Bolivian cities, the disease was uncontrolled. Reports included at least 8 human deaths and 965 rabid dogs.

In the city of Santa Cruz, rabies was diagnosed in 558 dogs and 4 humans. As a result, this was the highest occurrence of canine rabies in the Americas. During November 2017, a massive canine rabies vaccination campaign was organized. The program had the following characteristics: a) vaccination intensity was proportional to the risk; b) Both house-to-house and fixed-point vaccination was based on disease prevalence in time and space; and c) the measurement units included the homes and risk areas, which were considered as clusters. For this mass vaccination campaign, a cell culture vaccine was used.

The vaccination coverage was 74.7%, which was satisfactory to interrupt viral transmission. As a result, during 2018, 75 cases of canine rabies were reported in the city and only 3 canine rabies cases have been diagnosed up to July 2019. However, 4 cases of human rabies were recorded during the first quarter of 2018. These individuals were all exposed by rabid dogs during 2017. Subsequently, no more human rabies deaths were registered to date (July 2019). In summary, this strategy of immunization intensity and focus demonstrated the effectiveness of mass vaccination and the adequate use of resources to control this outbreak.

Effect of Antimalarial Drugs on the Immune Response to Intramuscular Rabies Vaccination Using a Post-Exposure Prophylaxis Regimen and the Immunogenicity and Proteomic Analysis of a Two versus Three doses, Intradermal and Intramuscular Administration of a

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Background: The WHO recommends ID administration of 3-doses of rabies vaccine for pre-exposure prophylaxis. Chloroquine is used for anti-malaria prophylaxis and is thought to impair rabies vaccine immunogenicity. We explored if antimalarials affect rabies antibody production and if 2-dose ID is equal to intramuscular (IM) administration. Methods: We conducted a study in 103 adults who received antimalarials 14 days prior to and during vaccination. In a second study we assigned adults to one of six treatment groups (ID vs. IM at 2 vs. 3-dose compared to controls). Results: In the first study, all subjects achieved protective neutralizing antibody titers of >0.5 IU/mL. We observed a reduction in rabies antibody titer in the chloroquine versus control groups 28 days after vaccination: 2.3 vs 6.87 IU/mL respectively ($p < 0.001$, t-test). A significant difference was not observed for those taking malarone or doxycycline (also anti-malarials). In the second study ID vaccination resulted in high antibody titers with all subjects achieving protective antibody levels (2 and 3-dose groups). At study day 365, protective levels were 40% for IM and 50% for ID (2-dose schedule); and 70% for IM, and 60% for ID (3-dose schedule). Conclusions: We observed no reduction of rabies antibody response in volunteers taking malarone or doxycycline, but a significant reduction in those taking chloroquine; however protective antibody levels were achieved in all groups. ID delivery of a rabies vaccine is equivalent to IM vaccination at a fraction of the dose concentration. These findings may impact rabies vaccine use recommendations.

ROUND TABLE
Import/Export Regulations
Moderator: Ryan Wallace

Forgeries of Rabies Serology Report for Pet Exportations

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With increasing international travel, the movement of companion animals has concomitantly increased. Though regulatory policies vary among jurisdictions, the majority of countries with rabies-free status require a minimum rabies antibody titer ≥ 0.50 IU/mL as proof of adequate vaccination. The Kansas State University (KSU) Rabies Laboratory is an OIE (World Organisation for Animal Health) approved laboratory that provides serological rabies testing for the purpose of pet importation. Antibodies to rabies virus are measured using Fluorescent Antibody Virus Neutralization and Rapid Fluorescent Focus Inhibition Test. Results are provided to owners on a serology report that serves as proof of rabies vaccination. A unique identifier ensures that results can also be accessed online by animal quarantine officials worldwide. CDC and KSU reviewed forged KSU serology reports identified by animal quarantine services and reported to KSU on a case-by-case basis.

We reviewed 81 cases of forged KSU serology reports notified by quarantine officials to KSU between 2006 and July 2019. Three quarters (n=61) of documents were for animals for which there was no record in the KSU database, 21% (17) were from animals tested at KSU but for which results were fraudulently altered, and 3 (4%) were unknown. Animals with forged records originated from more than 14 countries, primarily from Venezuela (n=37, 46%) and the United States (n=15, 19%). A majority of animals were bound for the European Union or European Economic Area (n=56, 69%). Of the 63 reports with available information, all forgeries involved dogs (69%) and cats (31%). Among the 17 altered reports, the date of serum draw and microchip number were the most frequently falsified variable.

Increased surveillance and collaboration between official rabies serology testing laboratories and animal quarantine services should be considered to prevent the illegal introduction of companion animals, and reduce the potential of introducing terrestrial rabies into rabies-free countries.

30th Rabies in the Americas – Kansas City, Missouri, United States of America – October 27 – October 31, 2019

Impact of an illegal importation: Heath response to an imported rabid dog—Kansas, 2019

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Background: On February 25, a dog imported from Egypt developed rabies; confirmed as canine rabies virus variant (CRVV) by CDC. This dog was imported with 25 other dogs by a pet rescue in a large metropolitan area. All had certificates of veterinary inspection and rabies vaccination.

Methods: Exposure risk assessments were conducted for persons and animals in contact with the rabid dog. Egyptian dogs were quarantined while prospective serological monitoring (PSM) was conducted by Kansas State University Rabies Laboratory to evaluate for evidence of previous vaccination (EPV). Length of quarantine was determined by PSM results. Vaccine manufacturers listed on rabies certificates were contacted to verify lot numbers.

Results: Forty-five people received rabies post-exposure prophylaxis. Twelve US dogs were exposed to rabies; two were unvaccinated and placed in six-month quarantine, 10 were administered booster vaccinations and observed 45 days. Of 25 Egyptian dogs; seven had EPV and were quarantined for four months, 18 had no EPV and were quarantined for six months. All dogs survived quarantine. Three vaccine manufacturers verified five lot numbers.

Conclusions: This is the third importation of a rabid dog from Egypt into the US in the past four years. Each imported case of CRVV risks re-introduction of the virus into the US and consumes public health resources. CDC issued temporary suspension of dogs entering the US from Egypt in May 2019 to prevent rabid dog importation. Permanent measures should require vaccinated dogs from Egypt to undergo PSM testing at an OIE-approved rabies laboratory and remain in quarantine until results are available.

ORAL PRESENTATIONS

Rabies Diagnostic Methods

Moderator: Christine Fehlner-Gardiner and Rolan Davis

30th Rabies in the Americas – Kansas City, Missouri, United States of America – October 27 – October 31, 2019

Development of a high throughput, low cost sequencing method for bat rabies virus and host identification using the Oxford Nanopore MinION sequencer

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Bats represent a diverse group of mammals with habitats extending to all continents except Antarctica. Several rabies virus (RABV) variants have evolved in America each associated with a different terrestrial mammal or bat reservoir species. In 2017, bats were the most frequently reported rabid wildlife animals in the USA with over 1400 bats testing positive according to the annual rabies surveillance report. Currently, genetic information among certain bat rabies virus variants is limited and uncertainty in identifying the associated bat hosts make it difficult to investigate transmission cycles and other aspects of bat rabies epidemiology. As part of ongoing efforts to improve rabies surveillance, we are developing a high throughput, low cost sequencing method using Oxford Nanopore technology. A multiple PCR approach was developed to generate PCR fragments covering the Leader sequence, Nucleoprotein (N) gene, Glycoprotein (G) gene and intergenic region between the G and Polymerase (L) genes of RABV genome as well the cytochrome B gene of the host. A large number of samples were mixed after labelling with specific sequence tags and sequenced using the Oxford Nanopore MinION sequencer. Seventy-five samples from rabid bats and twenty-nine samples from different terrestrial mammals were sequenced using this method. Rabies virus RNA from diverse bat and terrestrial rabies virus variants and cytochrome B from several diverse bat and terrestrial mammalian species were amplified by the multiplex PCR assay. The N, G and cytochrome B sequences provided detailed information on the distribution and associations of rabies virus and its hosts.

Low-cost rabies virus sequencing around the world using the Oxford Nanopore MinION

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For many countries with a high burden of canine rabies, control and eradication efforts are hampered by lack of information about the distribution and spread of rabies virus. Rapid, cost-effective sequencing could provide critical information about virus variants and virus-host relationships that is needed to inform control and vaccination strategies. The Oxford Nanopore MinION is a low-cost, portable sequencer that is poised for use in resource-limited locations. We evaluated the MinION as a portable option for sequencing in laboratories lacking the resources or expertise for sequencing. The sequencing strategy involved enrichment of rabies virus RNA from clinical and field samples using PCR amplification to detect and identify divergent rabies virus isolates. More than 200 rabies virus isolates were sequenced in Guatemala, India, Kenya, and Vietnam. Phylogenetic analyses provided valuable insight into rabies distribution in regions where rabies sequencing was not possible in the past, including uncovering a new rabies virus variant in Kenya and rabies importation into an Indian state after extensive vaccination and surveillance efforts. Taken together, our evaluation suggests the MinION can produce informative rabies sequences from clinical and field samples and highlight its potential for low-cost, portable sequencing of pathogens in locations without the ability to sequence.

Rapid, low cost rabies virus genetic typing using pan-lyssavirus real-time RT-PCR assay LN34 amplicon sequences

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Pan-lyssavirus real-time RT-PCR assay LN34 meets the requirements by the World Organisation for Animal Health (OIE) as a primary rabies diagnostic assay and has been used as a confirmatory rabies test in multiple laboratories. Through laboratory optimization and in silico comparisons, we developed an LN34 amplicon sequencing protocol for rapid, low cost genetic typing. The LN34 amplicon sequence is 165 bp in size, includes the Leader sequence and N-terminus of the Nucleoprotein (N) gene in the lyssavirus genome and is much cheaper to sequence than that of full/partial N gene. Although having lower resolution than that of N gene, over 100 rabies virus genotypes and other lyssaviruses species can be identified based on their unique sequence patterns using limited available rabies genome sequences. The LN34 genotypes correlated well with traditional rabies virus variant information. After testing positive by the LN34 assay, the LN34 amplicon sequence can be used to confirm the positive result, provide information about the geographic distribution of rabies virus variants, and narrow down the list of rabies cases that are highly similar and require additional genomic sequencing for phylogenetic studies. The LN34 PCR amplicon is highly stable and can be sequenced rapidly at a local laboratory or batched at a sequencing center. Using this method, laboratories use the LN34 assay for rabies diagnosis can implement genetic typing at little additional cost. This combined approach provides a practical way to improve rabies diagnostics and surveillance, an important step to achieve the goal of canine rabies eradication by 2030.

Rabies Diagnosis in the USA - Time for a Revision to the National Standard DFA Protocol?

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Over a decade ago, the National Working Group on Rabies Diagnosis proposed the Standard Protocol for DFA Testing for diagnosis of rabies in animals within the USA. Annually over 100,000 DFA tests are performed at 136 rabies laboratories. Data accumulated over 15 years indicates that less than 1% of these DFA tests are indeterminate after primary testing, and require confirmatory testing at the CDC. Although this is a small number of samples per day, due to the seriousness of the disease and potential fatal outcome, rapid and reliable confirmatory testing is required. Confirmatory testing is a repeat DFA with two anti-rabies FITC conjugates and a specificity control reagent (non-rabies FITC labeled antibodies). The anti-rabies conjugates available in the USA are limited to Fujirebio Diagnostics Cat# 800-092 (FDI), Millipore Sigma Cat#6500 and Cat#5500 [containing monoclonal antibodies (MABs) 502-2 and 103-7] and Millipore Sigma Cat#5100 containing three MABs. The specificity control, Millipore Sigma Cat#5102 (matches isotypes of the MABs in Cat#5100). Analysis of the samples received at CDC for confirmatory testing because of indeterminate primary DFAs indicates that greater than 99% demonstrate non-specific fluorescence in both specificity control 5102 and anti-rabies 5100 reagents, regardless if the samples are DFA positive or negative. In contrast, presence of non-specific fluorescence in problem samples received for confirmatory testing at CDC is less than 5% with FDI. We propose alternative confirmatory testing by antigen detection using DFA with one of the conjugates containing the 502-2/103-7 antibodies and simultaneous genomic RNA detection by LN34 real-time RT-PCR.

BioTek Cytation 5 and Gen5 software for high throughput analysis of rabies viral tests

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BioTek's Cytation 5 is a powerful tool for high throughput imaging and analysis. In this talk, I will describe the workflow for the automated preparation, imaging, and analysis of the plates for the FAVN and RFFT tests. The automated image acquisition and processing can read and analyze a 96-well plate in about 30 minutes with the accuracy of a human reader all while maintaining a visual record of each well (or sample) of the plate. Gen5 software is 21 CFR Part 11 compliant meeting the need of data security and integrity. Combined with a BioTek BioStack microplate stacker, with up to a 50 microplate capacity, the Cytation can imaging and analyze test results around the clock with minimal user interaction.

Preparation and Qualification of an Internal Rabies Reference Standard for RFFIT: From Present to Future

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The rapid fluorescent focus inhibition test (RFFIT) is the "gold standard" assay for measuring rabies virus neutralizing antibodies (RVNA) in sera. The World Health Organization (WHO) encourages standardization across multiple RFFIT assay formats by generating WHO-1 and WHO-2 standard rabies immune globulins (SRIGs), the stocks of which are no longer available. This study sought to establish an internal rabies reference standard (IRRS) by: (1) using human rabies immunoglobulin produced by Sanofi Pasteur (designated IMORAB2) and (2) pooling serum samples obtained from healthy adults immunized with licensed rabies vaccine (GCIRAB1). For both, bulk material was prepared by diluting with serum-free cell culture medium and candidate IRRS was qualified for use in RFFIT according to established procedures (a). The unitage (IU/mL) was assigned by testing candidate IRRS in multiple assay runs by different analysts to generate >/= 50 results that were assessed and calibrated against WHO-1 and WHO-2 SRIGs. The observed geometric mean concentration (GMC) for IMORAB2 calibrated against WHO-1 and WHO-2 was 1.8 IU/mL and 1.5 IU/mL (precision 18.7% and 17.8%, respectively). The observed GMC for GCIRAB1 calibrated against WHO-1 and WHO-2 was 2.9 IU/mL and 2.5 IU/mL (precision 17.5% and 16.7%, respectively). Candidate IRRS specificity was demonstrated in competition study using homologous and heterologous antigens, accuracy/linearity for WHO-1 and WHO-2 SRIGs using candidate IRRSs was acceptable, RFFIT LLOQ of 0.2 IU/mL was confirmed, and concordance between IRRS and WHO SRIG was demonstrated. The results showed that the candidate reference materials are suitable for use as IRRS in the rabies RFFIT.

(a) Timiryasova TM et al. Rapid Fluorescent Focus Inhibition Test Optimization and Validation: Improved Detection of Neutralizing Antibodies to Rabies Virus. J Immunol Methods. Jun 2019. [Epub ahead of print]. doi: 10.1016/j.jim.2019.06.017.

ORAL PRESENTATIONS

Spatial and Economic Disease Modeling

Moderator: Ram Raghaven and Ryan Wallace

Updating scenarios for human rabies in the Peruvian Amazon Basin

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Since 2011, Peru started a public policy to administer rabies pre-exposure prophylaxis (PreEP) to all population living in high-risk areas for vampire bat rabies. The success of implementing this policy is evidenced in lack of human cases among population immunized. Almost a decade after the start of the intervention, there is still extensive areas of the Peruvian Amazon Basin (PAB) to cover. Significant changes in population size, domestic animal population, economic activity, migration and communications are observed in some parts of the PAB, but without noticeable improvement of healthcare access. To account for those changes and the impact of the massive PreEP interventions, this study revisits the rabies risk models used a decade ago to estimate populations at risk and outline the new updated rabies risk situation for the PAB. The models used geographic data, immunological evidence, epidemiological surveillance, and economic data. While population at risk had been reduced with correct prioritization in the order of the places to intervene; the slow advance of the PAB coverage was due to predictable factors related to outreach logistics in challenging topography, and also to unexpected factors such a governance blocks, budget delays, political uncertainty and others related to local governments. The insights from the current rabies risk scenarios in the PAB are of interest for the ongoing implementation of massive PreEP policies in neighboring Amazon Basin countries.

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Rabies surveillance identifies potential risk corridors and enables management evaluation

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Intensive efforts are being made to eliminate the raccoon variant of rabies virus (RABV) from the eastern United States and Canada. The primary method to manage RABV in wild populations is through the use of oral rabies vaccination (ORV) to reduce the susceptible portion of the target population and consequently reduce and eliminate RABV transmission. The National Rabies Management Program has implemented enhanced rabies surveillance (ERS) to improve case detection across the extent of the raccoon (ORV) management area. We used a dynamic occupancy approach to examine ERS data from 2006-2017 in three northeastern U.S. states to examine potential risk corridors for RABV incursion from the U.S. into Canada, evaluate the effectiveness of ORV management strategies, and identify surveillance gaps. ORV management has resulted in a decrease in RABV occupancy over time within management zones compared to stable RABV occupancy probabilities south of ORV management areas. Although RABV occupancy was related to habitat characteristics, greater impacts were associated with ORV activities and seasonal and yearly trends. ORV management in this region began as early as 1995 and the ORV zones were modified yearly; therefore management continuity varied across the study area. During the first five continuous years of ORV baiting in an area, dramatic decreases in RABV occupancy probabilities were observed. Further reductions in RABV occurrence were observed again after 12 years of continuous baiting, which corresponded with a change in baiting strategy. We identify potential risk corridors, management impact over time, and surveillance needs to help inform future management.

EXPLORING THE EFFECTS OF ARCTIC FOX ECOLOGY ON RABIES EPIDEMIOLOGY IN NORTHERM QUEBEC USING A SPATIALLY-EXPLICIT INDIVIDUAL-BASED MODEL

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Arctic rabies is a health threat to human populations and domestic animals in northern polar areas. Arctic rabies persists in its main reservoir species, the Arctic fox (Vulpes lagopus), despite its low population density. Our understanding of the dynamics of rabies epidemiology remains limited, in part, due to the difficulty of obtaining ecological and surveillance data from remote and expansive regions. Knowing where to monitor the emergence of new cases of rabies is a challenge for disease management. The incidence of rabies in the Canadian Arctic varies in space and time. The variation in space may be because two main northern ecotypes (inland, coastal) that influence fox density, movement and interaction. Inland foxes feed primarily on lemming populations which provide a multi-year cyclical resource. Coastal foxes feed mainly on marine resources (e.g. marine animal carcasses) and this provides a more constant resource. Here we use a spatially-explicit individual based model to test how the spatial and temporal structures of the inland and coastal ecotypes affect rabies dynamics given their influence of arctic fox density, movement and interactions. The results indicate that the dynamics of the disease develop with varying intensities and speeds depending on the spatio-temporal structures of the resources. Our modeling approach allows us to identify areas where the disease is transmitted more quickly, information which can be used to more effectively target surveillance.

Dog movement analysis associated with a rabies outbreak response on the Haiti-Dominican Republic border, 2019

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During 2019 three dog-mediated human rabies cases were identified in the town of Pedernales, Dominican Republic, which shares a border with Anse-a-Pitre, Haiti. Many residents associated the rabies outbreak in Pedernales with unvaccinated dogs coming across the border from Haiti. During January and February of 2019, a multi-national team from Haiti, the Dominican Republic, and the United States responded to the outbreak and evaluate the possibility of dog border crossing as a potential source. Visual inspection of the area found multiple sources of food from waste dumps and markets directly adjacent to the international border. Dogs marked during vaccination activities in Haiti were also observed by team members in the Dominican Republic. A sample of five dogs were fitted with GPS collars and had their movements recorded every 5 minutes for 48 hours. The majority of dogs stayed close to their residence for the entire period. One dog was recorded on both sides of the international border. Additional analysis of dog movement patterns in Haiti would be beneficial for understanding rabies transmission risk.

ORAL PRESENTATIONS

Community Engagement

Moderator: Elizabeth McQuade and Peter Costa

30th Rabies in the Americas – Kansas City, Missouri, United States of America – October 27 – October 31, 2019

Rabies Management in the North. Analysis of Alaska, Northwest Territories and Svalbard.

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Apart from logistical challenges such as limited physical and administrative infrastructure and remote location, rabies management in the North also falls under multiple administrative jurisdictions as it poses a threat to human health but is largely managed through prevention efforts in animals. I compared rabies management in Alaska, Northwest Territories and Norway (Svalbard archipelago) using semi-structured interviews and document review. Approaches and challenges in rabies management differed among the three cases of this study. While remoteness, dog population management, and dog vaccination are of main concern in Alaska and Northwest Territories these issues were not mentioned by public health officials in Norway. On the other hand safety of reindeer hunters was a concern only in Norway. In all cases the rulemaking authority largely rests with regional or local officials while national public health institutions provide crucial technical expertise and support in the form of diagnostic testing, epidemiological support, and expert advise. In this presentation I will discuss these differences in the context of One Health that combines human animal and environmental health in a multidisciplinary approach but poses significant administrative challenges interagency collaborations.

Raising awareness about bat rabies in the Americas through a One Health approach

Peter Costa

Peter Costa, Clarissa Noureddine, Sara Reilly De Wet, Juliana Galhardo, Jennifer Diethelm, Jennifer Hulsey, Malathi Raghavan, Robert Blew, Bernadette Dunham, Karen Gruszynski, Bonnie Price, Janine Seetahal, Tariku Jibat Beye, and Cheryl Stroud

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The One Health Commission Bat Rabies Education Team (OHC BRET) was founded in 2016 with the mission to raise awareness about bat rabies in the Americas by promoting education in a multi-strategic One Health approach. Comprised entirely of dedicated volunteers, BRET was created because there seemed to be no focused health communication efforts in the Americas to educate the public that bats can be infected with and transmit the deadly rabies virus. Existing educational initiatives include the launching and constant updating of the BRET website, which serves as a hub for bat rabies information; and educational posters in English, Spanish and Portuguese developed in partnership between One Health Commission, Global Alliance for Rabies Control and Bat Conservation International. Throughout all BRET resources, messaging focuses on not harming bats, not touching bats and to seek medical advice if contact with a bat occurs. Recently BRET surveyed public health professionals in the Americas to discover what, if any, bat rabies educational initiatives exist. Of the 228 international respondents from human, animal and environmental health sectors, 55.3% indicated "No" or "Don't Know" when asked if their agency has developed any resources specifically for bat rabies education. The need for education bat rabies education in the Americas is immense and the OHC BRET is pleased to be able to provide a library of free, downloadable resources and to spearhead communications about protecting bats while recognizing the potential for them to transmit rabies to humans and pets. For more information, please contact batrabies@onehealthcommission.org.

CONTENCIÓN DE LA RABIA URBANA BASADA EN LA ATENCIÓN PRIMARIA A LA SALUD, EN EL ESTADO DE PUEBLA

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The implementation of successful strategies in the context of rabies must be comprehensive and inserted into an appropriate Prevention, Control and Surveillance Program, which remains alert to meet a common goal.

In Puebla the vision is clear, the absence of anger in the dog and the cat, and therefore in the human. Strategies based on the coordination-cooperation of the different levels of government, institutions and the participation of a conscientious and informed society are encouraged, which are driven towards the same direction and benefit, that of Una Salud; therefore, canine and feline rabies vaccination, as well as the surgical sterilization of these species make it evident and in figures, that in Puebla the results are tangible, 0 cases of rabies in dogs and cats and 0 deaths due to rabies for 10 and 20 years respectively.

Just like a gear, these actions require for their execution, the management and support from the local area, that is, to encourage from the health houses to maintain the achievement and gradually promote the containment of rabies.

With the above, what is suggested in the Comprehensive Health Services Networks (RISS), which encourage community activation and participation in neglected diseases, such as rabies, is exhausted.

The co-responsibility between the instances as municipalities and health institutions, with a commitment from the budgetary issue to the operational, is essential to feed back with maximum frequency, to ensure the coherence of these actions. "

"Zero by 30" - should we really be concerned about wildlife?

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Rabies is not a candidate for true eradication, due in part to the diversity of mammalian reservoirs, especially bats. This zoonosis may be prevented by avoiding viral exposure and prompt, appropriate prophylaxis, once exposure occurs. Mass vaccination of domestic dogs is the most significant method to minimize the disease burden. Canine rabies has been eliminated in all developed countries and is increasingly controlled in lesser developed countries (LDC). Recognizing the utility of both public health and veterinary strategies, the global elimination of human rabies via dogs (GEHRD) is set for 2030. Although controversial, management of wildlife rabies reservoirs should be considered integral for successful implementation of the GEHRD. Among wild carnivores, the canids represent one of the most important mammalian groups, regarding rabies perpetuation. Significant genera in this family include: Canis; Nyctereutes; Urocyon; and Vulpes, among others. All known canid reservoirs maintain the perpetuation of domestic dog rabies virus variants. Jackals in Eurasia and Africa, coyotes in North America and several other taxa throughout the Americas are all vector-competent. Appreciation of the role of these or other major carnivores associated with human rabies cases (such as ferret badgers, mongooses, etc.) is often lacking in LDC. To date, oral rabies vaccination (ORV) programs targeting several wild carnivore species have successfully managed rabies in Eurasia and North America. Given the time needed to develop additional modern biologics, attractive baits and effective ORV strategies, such knowledge about the importance of wildlife to maintenance of rabies is critical in the short term. Although the GEHRD may achieve the major portion of its goal by focusing upon humans and domestic dogs alone, enhanced laboratory-based surveillance, pathogen characterization and refined epizootiological insights on wildlife rabies are necessary, if the program is to achieve true success over the next decade.

ROUND TABLE

Rabies Serology and Vaccinations

Moderator: Susan Moore

Rabies serology: Issues in its role in the prevention and control of rabies

Susan M. Moore

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Rabies pathogenesis, epidemiology, and immunology all play a role in designing effective strategies for control and prevention of rabies in both an individual and a population. The fact that it is a zoonotic disease with the highest case fatality rate of any infectious disease demands establishment of strict laws, clear guidance, and reliable surveillance for disease prevention. The need for mandatory rabies vaccination in pets remains to provide protection for both pets and humans. The anti-vaccine movement has gained popularity among pet owners. Some veterinarians and pet owners have proposed use of reduced dose rabies vaccination, as well as use of rabies serology as proof of rabies immunity to avoid booster vaccination. Forgeries of rabies serology reports for pet import/export are not uncommon. In addition, use of rabies serology to prove previous vaccination or exposure is applied in various ways. Currently, there is no standard recommended rabies serology method or method associated acceptance level for all purposes of rabies serology. Establishment and practice of important considerations for fit for purpose include appropriate method, quality control/assurance (including proficiency testing), global standardization across groups involved in surveillance (including reagents/kits), and sharing of expertise. This round table will discuss current efforts and information to aid in obtaining the best quality rabies serology data for research, public health and medical/veterinary decisions.

30th Rabies in the Americas – Kansas City, Missouri, United States of America – October 27 – October 31, 2019

Proficiency test for rabies serology: a design complying with international standards for a reliable assessment of participating laboratories

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Since 1993, many rabies free countries have alleviated their quarantine measures and adopted a scheme requiring animal identification and rabies vaccination followed by a serological control, neutralising antibodies being the most reliable indicator of successful vaccination. This alternative measure allows guaranteeing the safety of free movements of pets and preserves the rabies free status of the countries.

For pets moved into the European Union, the European Commission decided to establish a system of community approval of laboratories willing to carry out the rabies serological controls in order to guarantee an effective control system. As the specific institute to coordinate the approval of the laboratories, designated by the European Commission in 2000, our main task is to organize an annual rabies serology proficiency test for laboratories already agreed or willing to be agreed to perform rabies serological controls in the frame of international trade of pets.

To guarantee a reliable way of performances' assessment to participants, we have adapted our process to comply with international Standards. The major challenge has been to find an adequate statistical approach complying with the Standards while staying in line with the purpose of this proficiency test.

The description of the rabies serology proficiency tests including the statistical approach used for analyzing participants' data as well as a complementary analysis of data obtained from participants will be presented.

Owner reported rabies vaccination and serologic response to vaccination in working equids in the Peruvian Andes

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Vampire bats prey on livestock and humans in Peru and act as a reservoir for rabies virus. Rabies vaccination is recommended to protect animal and human health. We aimed to assess rabies virus neutralizing antibody (RVNA) titers in unvaccinated and vaccinated populations of working equids in the Peruvian Andes.

Blood samples were collected from a convenience sample of 288 horses, mules and donkeys during a veterinary field clinic. Serum RVNA titer was measured by use of a rapid fluorescent focus inhibition test. Owners reported prior vaccination and bat exposure.

Vaccine history was available for 200/288 sampled equids, and 65% of these animals (130/200) received at least one rabies vaccination. RVNA titers (>0.5 IU/mL) were significantly higher in vaccinated than unvaccinated animals (77% vs. 19%; $p < 0.01$). 37% of animals with one vaccination (10/27) had RVNA values <0.5. Bat exposure data was available for 177 equids with known vaccination status with 47% (n=84) exposed. In unvaccinated animals, seroprevalence was higher (31%; 4/13) in exposed equids versus unexposed (17%; 7/41). RVNA values ranged from <0.1 - 140 IU/mL.

Low (<0.5) or undetected (<0.1) RVNA titers represent equids that are unvaccinated, vaccinated once, vaccinated a long time ago, or poor responders. Protection may be inadequate following primary vaccination with one dose. Equids with detectable RVNA titers that were reported to be unvaccinated but exposed to bats (n=4) may have titers from direct exposure to rabies virus from bat feedings. Vaccination of equids in rabies endemic areas is paramount.

Tailoring vaccine protocols to benefit the patient and practice

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More and more pet owners are starting to question veterinarians and their staff about the need for vaccinating their pets due to misinformation on the internet, as well as sensationalized stories about pets who may, or may not, have experienced a vaccine reaction. Those in opposition of vaccines are passionate about their views, while those in favor are equally passionate on the subject. Is one side right and the other wrong, or is there common ground to be found? Likely a little of both. Not all is yet known about the immune system, and there is still research that needs to be done. As such, recommendations offered today are based on current information, with the full knowledge they could change in the future. Vaccine opponents often argue ingredients such as adjuvants (e.g. aluminum, mercury, formaldehyde, and foreign proteins) are reasons to not vaccinate, as they can be the cause of adverse advents. This was true in the past; however, many vaccines have been "purified" over the years through the removal of extraneous proteins. These changes in production have made vaccines much less reactive for our patients over the years. Vaccines are safe, effective, and recommended for the majority of patients, are there times when they can be harmful for some? While adverse reactions to vaccination can occur in many species, the rate of these reactions is low. The risk of not having immunity to common infectious organisms far outweighs the threat of developing serious illness as a result of vaccination.

ROUND TABLE
Skunk Rabies
Moderator: Amy Gilbert

30th Rabies in the Americas – Kansas City, Missouri, United States of America – October 27 – October 31, 2019

Why are skunks so susceptible to several rabies virus variants?

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Skunks, members of the Mephitidae mammalian family, are distributed throughout the Americas and are notable for their ability to harbour many different and phylogenetically distinct rabies virus variants. In the USA and Canada the striped skunk has traditionally been the primary reservoir host for three viral variants: the south central skunk (SCSK), the north central skunk (NCSK), also referred to in Canada as the Western skunk (WSK), and the Californian skunk (CASK). Additional rabies virus variants associated with other skunk species have been described in Mexico. However skunks are also notable for their susceptibility to infection by other rabies virus variants and they often constitute the main "spill-over" species for rabies viruses associated with other hosts such as raccoons and foxes. The frequent infection of skunks by the raccoon rabies virus variant has prompted suggestions that the skunk acts as a secondary reservoir host for this variant in some jurisdictions. Moreover there are two documented instances of rabies virus host switching, from bat and fox reservoirs respectively, into skunks. While ecological factors likely contribute significantly to the skunk's role as a rabies reservoir, sequencing studies have explored whether there could be features of the viral genome that facilitate the virus' adaptation to and persistence in this host. Knowledge in this area will be presented.

Oral rabies vaccination of wildlife: What drives vaccine uptake efficiency in different reservoir species?

Verena te Kamp, Conrad M. Freuling, Ad Vos, Peter Schuster, Christian Kaiser, Steffen Ortmann, Antje Kretzschmar, Sabine Nemitz, Elisa Eggerbauer, Reiner Ulrich, Jan Schinköthe, Tobias Nolden, Thomas Müller, Stefan Finke

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Oral rabies vaccination (ORV) is highly effective in foxes and raccoon dogs, whereas for unknown reasons, the efficacy of ORV in other reservoir species is less pronounced. To investigate possible variations in species-specific cell tropism and local replication of vaccine virus, different reservoir species were orally immunized with a highly attenuated, GFP-expressing RABV. Clear differences among species were observed suggesting host specific limitations to ORV. While for responsive species the palatine tonsils was identified as a main site of virus replication, less virus dissemination was observed in tonsils in rather refractory species. Although the comparison of vaccine virus tropism would suggest a direct correlation of tonsil infection and responsiveness to ORV, our data indicate that lymphoid tissues other than the palatine tonsil may play a more important role in eliciting an immune response as originally anticipated. Overall, these data support a model in which the susceptibility to oral live RABV vaccine infection of lymphatic tissue is a major determinant in vaccination efficiency. The present results may help to direct future research for improving vaccine uptake and efficacy of oral rabies vaccines under field conditions.

Evaluation of ONRAB at low and then high bait density applications in West Virginia, USA

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Rabies managers in the United States (US) have been looking at expanding vaccine-bait options available for oral rabies vaccination (ORV). One product undergoing field evaluation is ONRAB, a recombinant human adenovirus-rabies glycoprotein vaccine. The first ONRAB field trial in the US occurred in 2011 in West Virginia resulting in a rabies virus neutralizing antibody (RVNA) seroprevalence of 49% in raccoons (Procyon lotor) and 7% in skunks (primarily striped skunks [Mephitis mephitis]). Field trials continued in West Virginia in 2012-2013 using a bait density of 75 baits/km^2 followed by three years at 300 baits/km^2 to target raccoon rabies spillover cases persisting in skunks. We evaluated changes in the raccoon and skunk population RVNA seroprevalence during 2012-2016, focusing on the impact of increasing bait density. For raccoons, the average seroprevalence increased post-baiting from 53% at 75 baits/km^2 to 82% at 300 baits/km^2. For skunks, the average seroprevalence increased post-baiting from 13% at 75 baits/km^2 to 40% at 300 baits/km^2, peaking at 59% in 2016. In both species, the pre-baiting seroprevalance demonstrated an increasing trend across the study years. The lower bait density may suffice for controlling rabies virus transmission in raccoons, but the higher bait density may be needed for targeting skunks. Multiple years of baiting helps to maintain higher residual levels of RVNA in the population, ultimately enhancing the ability to halt rabies virus transmission in target host populations. This study allows managers to refine ORV to better target both raccoons and skunks in an effort to eliminate raccoon rabies.

Volatile metabolomic signatures of rabies immunization and infection in skunks and raccoons

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Rabies virus circulates naturally in several wild carnivore reservoirs in the United States, some of which are also targets of oral rabies vaccination (ORV) control efforts. Two vaccine efficacy studies conducted with striped skunks (Mephitis mephitis) and raccoons (Procyon lotor) provided an opportunity to determine whether volatile fecal metabolites might be used to non-invasively monitor ORV programs and/or predict virus protection for these species. Fecal samples were collected at multiple time points from raccoons and striped skunks subjected to oral vaccination as well as an intramuscular challenge with a lethal dose of rabies virus. In addition to fecal samples, blood was collected at multiple time points to quantitatively assess rabies antibody responses arising from immunization and/or infection. Feces were analyzed by headspace gas chromatography with mass spectrometric detection and the chromatographic responses were subjected to repeated measures analyses of variance (ANOVA) to identify fecal odorants associated with immunization and/or virus challenge. Multiple regression was then used to model and predict quantitative immune responses from the metabolomic data. The ANOVA results identified compounds associated with vaccination of skunks, but the volatile metabolites tended to vary greatly over the post-vaccination period. Conversely, regression models demonstrated considerably greater success in predicting rabies antibody responses in both species, regardless of elapsed time since immunization. This is the first study to link volatile compounds with measures of adaptive immunity and provides further evidence that the volatile metabolome holds great promise for contributing to our understanding of disease and infections.

Lightning Talks

Moderator: Mylissia Smith and Rodney Rohde

Introduction of a mobile solution to Integrated Bite Case Management – the REACT App

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Since the introduction of the global goal to eliminate dog bite transmitted human rabies by 2030, rabies control has gained traction around the world. However, to date, rabies control programmes often lack robust surveillance systems to both determine the actual burden of disease in a target population, as well as tracking the impact and progress of control programmes.

The Integrated Bite Case Management (IBCM) was developed to address this need for better rabies surveillance in line with WHO's call for Integrated Disease Surveillance and Response. Successfully implemented by the government of Haiti in collaboration with the US Centers for Disease Control and Prevention (CDC), IBCM now serves as the blueprint for a rabies surveillance system that does not only collect the data needed to assess the burden of disease but also enables national public health systems to distribute resources in the most effective and economical way. Through its complexity, IBCM implementation faces many challenges. Following the complete algorithm and feeding the records into a national database for further analysis have been identified as some of the biggest hurdles to overcome.

In collaboration with the CDC, Mission Rabies and WVS have developed the Rabies Epidemiology And Case Tracker application, enabling IBCM data to be collected in the field on a smartphone in near real-time. Currently being used on a large scale in 3 countries, the REACT app provides a simple, quick and accessible solution to rabies surveillance, helping to reduce the burden of rabies and the efficient use of rabies vaccines.

Inactivated Rabies Virus-Vectored Immunocontraceptive Vaccine in a Thermo-Responsive Hydrogel Induces High and Persistent Antibodies against Rabies, but Insufficient Antibodies against Gonadotropin-Releasing Hormone for Contraception

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Rabies is preventable through vaccination, but the need to mount annual canine vaccination campaigns presents major challenges in rabies control and prevention. The development of a rabies vaccine that ensures lifelong immunity and animal population management in one dose could be extremely advantageous. A nonsurgical alternative to spay/neuter is a high priority for animal welfare, but irreversible infertility in one dose has not been achieved. Towards this goal, we developed a rabies virus-vectored immunocontraceptive vaccine ERA-2GnRH, which protected against rabies virus challenge and induced >80% infertility in mice after three doses in a live, liquid-vaccine formulation (Wu et al., 2014). To improve safety and use, we formulated an inactivated vaccine in a thermo-responsive chitosan hydrogel for one-dose delivery and studied the immune responses in mice. The hydrogel did not cause any injection site reactions, and the killed ERA-2GnRH vaccine induced high and persistent rabies virus neutralizing antibodies (rVNA) in mice. The rVNA in the hydrogel group reached an average of 327.40 IU/mL, more than 200 times higher than the liquid vaccine alone. The Gonadotropin-releasing hormone (GnRH) antibodies were also present and lasted longer in the hydrogel group, but did not prevent fertility in mice, reflecting a possible threshold level of GnRH antibodies for contraception. In conclusion, the hydrogel facilitated a high and long-lasting immunity, and ERA-2GnRH is a promising dual vaccine candidate. Future studies will focus on rabies protection in target species and improving the anti-GnRH response.

Oral vaccination of the small Indian mongoose against rabies: bait design optimizing vaccine release in the oral cavity

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Recent studies have shown that the small Indian mongoose can be vaccinated successfully by the oral route with the 3rd generation oral rabies virus vaccine, SPBN GASGAS. Safety of this vaccine construct has been shown for this target species and has been demonstrated in relevant non-target species as well. A highly attractive bait that has showed high bait acceptance by small Indian mongooses under experimental and field conditions has been developed. The remaining challenge to achieve effective vaccination is to secure reliable and efficient vaccine release in the oral cavity, which is the subject of current studies. This aim is complicated given the relatively small size of this carnivore and the fact that the animal sometimes uses its forepaws to fixate food items. In this study, the highly attractive bait matrix was fixed (egg-flavoured), with different sachet variants being the main variable investigated, namely the size and material used. In addition, the effects of a warning label was examined; with or without label and, if so, its position. Some of the sachets were covered with a special foil to increase bait matrix adherence; the effect of the presence of this foil on the immune response was also investigated. Finally, several variants of the bait matrix were explored; thick or thin layered, one – or two-sided. It seems that the effect of individual variation in bait handling on the successful release of the vaccine virus in the oral cavity of this animal species is much more pronounced in comparison to larger animals like red foxes.

ORAL PRESENTATIONS

Vaccines and Theraputics

Moderator: Alejandro Parola and Chandra Gordon

A dual-target rabies/yellow fever vaccine candidate

Lorena Sanchez-Felipe, Lotte Coelmont, Niraj Mishra,Sanne Terryn, Hermann Giresse Tima, Pauline Lehebel-Percier, Sapna Sharma, Guanghui Wu, David Selden, Marta Romano, Ashley C. Banyard, Anthony R. Fooks, Steven Van Gucht, Johan Neyts and Kai Dallmeier

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Human rabies vaccines require multiple doses, a cold-chain and involve high costs. A higher coverage may be reached with a cheap, thermostable and potent (one-dose) prophylactic vaccine. To this end, we developed a dual rabies/yellow fever vaccine using our PLLAV (Plasmid-Launched Live-Attenuated-vaccine) technology (WO/2014/174078) and the live-attenuated yellow fever vaccine strain (YF17D/Stamaril®) as viral vector. We succeeded in engineering a transgenic YF17D vaccine in which the major immunogenic rabies antigen (RabG) was inserted. The E/NS1 intergenic region was identified to be the most optimal integration site for this purpose. A short deletion of RabG was required to result in a construct from which viable live-attenuated viruses can be launched. This expresses immunogenic RabG in the context of replicating YF17D. The resulting transgenic virus is stable in cell culture for at least 8 passages. A single dose of YF17D/rabies injected in (interferon-deficient) AG129 as well as wild-type Balb/c mice consistently results in the induction of long-lasting virus-neutralizing antibodies and vigorous T-cell responses against both rabies and YF. Efficient dual protection against lethal challenge with either rabies or YF challenge was demonstrated in mice. Despite being highly immunogenic, YF17D/rabies is ~10.000-fold attenuated over YF17D/Stamaril® when inoculated in the brain of mouse pups. In conclusion, YF17D-RabG is a promising bivalent vaccine candidate that may be developed for childhood vaccination in regions where both viruses are prevalent.

This project has received funding from the European Union's Horizon 2020 research and innovation program under RABYD-VAX grant agreement No 733176.

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Rabies vaccination trials in common vampire bats using a raccoonpox recombinant vaccine.

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In Latin America, vampire-associated rabies is a public health concern and a burden to the livestock industry. Current control methods -culling vampire bats- should be revised and updated. As an alternative approach, we are evaluating a recombinant vaccine that uses a raccoon pox viral vector (RCN) expressing a mosaic glycoprotein gene (MoG) designed to provide broader antigenic coverage than currently available rabies vaccines. Ninety-three common vampire bats were captured in northern México and transported for our study to Madison, Wisconsin. Bats were tested for presence of neutralizing antibodies using the micro-RFFIT and based on these results, allocated into different groups for vaccine efficacy experiments: 1) seronegative males, 2) seropositive males, or 3) combined females. Groups were vaccinated orally or topically using glycerin jelly as a vehicle, controls were sham vaccinated. Some males from the seronegative group were boosted on day 100 post-vaccination. Blood samples were collected twice post-vaccination and prior to challenge. Bats were challenged using a coyote strain of RABV and observed for 7 weeks for clinical signs of rabies then humanely euthanized and sampled for rabies. All but one seropositive bat at baseline survived challenge. Rabies mortality after challenge was higher in seronegative males than seronegative females. Seven pups born during the experiment (< 3 mo. old) were also challenged; all succumbed to rabies and showed clinical signs. Antibody response post-vaccination and challenge will be discussed. During the study, rabies occurred pre-challenge in vampire bats as late as 210 days after capture. A vampire bat-associated strain of rabies was isolated and sequenced from afflicted bats.

Anti-rabies compounds developed by PiPiQ, a new drug discovery technology

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A new anti-rabies compound TCB-025 was designed and synthesized using a new drug discovery technology, Peptidomimetics for Inhibition of Protein-protein Interactions using CUbic scaffolds (PiPiQ). There is no effective treatment for rabies after onset of clinical symptoms, and development of a new drug is desired. In this study, we applied alanine-scanning method to the rabies nucleoprotein and found that a very short amino-acid sequence in the intrinsically disordered protein (IDP) region is essential for the lifecycle of rabies virus (RABV). This IDP region takes a helical conformation in the nucleoprotein-RNA complex (PDB: 2gtt) and forms a convex surface to interact with another nucleoprotein. The convex motif in nucleoprotein is composed of only five amino acids and expected to be a druggable target by small-molecule peptidomimetics. However, the conventional planar non-peptidic PPI inhibitors can't reproduce the three-dimensional orientation of the five amino acids. For the purpose of enabling provision of three-dimensional PPI inhibitors, we developed a new technology, PiPiQ where the coordination of amino-acid residues at the PPI interface are fixed covalently on a cubic scaffold of small molecule. Here we adopted an alkaloid skeleton as the cubic scaffold. Combining PiPiQ and structure-based drug design (SBDD), we succeeded in very efficient hit finding to obtain TCB-025 as an active compound with an IC50 of about 5 μM against a street RABV strain in vitro. Moreover, TCB-025 efficiently suppressed RABV replication in infected mice. Further structural optimization gave a 10-times higher active compound and its evaluation in mice is currently undergoing.

Preliminary experimental evaluation of Griffithsin's effectiveness against rabies virus infection in a hamster model.

Nadia F. Gallardo-Romero, Chantal Kling, Barry R. O'Keefe, Kenneth E. Palmer, Claire Godino, Felix Jackson, Lauren Haugh-Krumpe, Divyasha Saxena, Laurie Seigler, Andres Velasco-Villa and Christina L. Hutson.

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The current post-exposure prophylaxis (PEP) schedule includes the use of rabies immune globulin (RIG) in combination with four doses of vaccine. This treatment is effective if received before the onset of neurological signs. However, RIG makes PEP very expensive and often unaffordable in resource-limited countries where rabies has its greatest impact in human populations, with approximately 59,000 human deaths annually. The lectin protein Griffithsin (GRFT) has been widely studied and tested against several RNA viruses, which use a glycoprotein to infect host cells. In this study, we first assessed GRFT's rabies virus neutralization activity in vitro finding complete neutralization at concentrations of 200, 40, 8 micrograms/mL. When tested in the hamster model, GRFT (both 5 and 10 mg/kg doses) used in combination with vaccine (started on day 4 post inoculation), resulted in 60% survivorship compared to just 20% for the vaccine only against a lethal rabies challenge. RIG combined with vaccine was still the optimal treatment, resulting in 100% survivors. GRFT was detected in the brain of these hamsters at concentrations of 6.6 ng/ml per gram of tissue, 24 h post treatment, which indicates GRFT passed the blood-brain barrier. In addition, we noted the RABV neutralizing activity in sera from all GRFT treated animals was significantly higher than untreated animals (up to 331.4 IU/mL in some individuals, compared to 0.01 IU/mL in the HRIG group). The preliminary data obtained in this study warrants more robust experiments to gauge the effectiveness of GRFT against RABV.

ORAL PRESENTATIONS

Human Rabies Treatment and Prophylaxis

Moderator: Rodney Willoughby and Charles Rupprecht

30th Rabies in the Americas – Kansas City, Missouri, United States of America – October 27 – October 31, 2019

EVOLUTION OF THE CLINICAL PICTURE IN CASES OF HUMAN RABIES IN MEXICO IN THE PERIOD 2000-2018

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Since 2000 to date 52 human rabies cases have been registered in Mexico. From those, 7.7% (4 deaths) were originated by dogs and the remaining 92.3% (48 cases) were linked to wild species (chiroptera, skunks and foxes). Males are mostly affected with 59.6% (31) of cases, also children (less than 15 years old) with 57.7%.

None of the cases requested timely medical or rabies care, due to ignorance of other transmitting species, and presented a clinical evolution of general malaise in 75% (39), paresthesia (50%), fever (46%) and hyperirritability (46%), with Central Nervous System involvement, entering second or third level Hospitals.

The most common incubation period was two months (40% of cases), according to the aggressor species (dog 141 days, skunk 75 and bat 31 days). By site of inoculation: thoracic limbs (40%) in 39 days and head 31 (25% of cases) and the clinical evolution, which in recent years (2016 and 2018) has started with abdominal pain (confused with gastrointestinal pathology).

Once the prodrome was established, the status period was of 10 days average (foxes and bats).

The characteristic triad (hydrophobia, aerophobia and photophobia) was reported in three deaths (5.7%), hydrophobia with aerophobia in 10 (19.2%), aerophobia with photophobia in 5 (9.6%) and hydrophobia with photophobia in 4 (7.7%).

Variants in the clinical evolution are important for the physicians to identify a case of rabies among other diagnoses, linked with aggression or contact with this fauna and thus diagnose this disease.

Challenges of human rabies in Brazil in the 21st century: analysis of the epidemiological profile from 2000 to 2017

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Background: Despite advances in the prevention of human rabies, sporadic cases occur in Brazil, being an important public health problem. Objectives: to describe the epidemiological profile of human rabies in Brazil, 2000-2017. Methodology: a series of cases of human rabies reported 2000-2017, with a spatio-temporal distribution. Results: 188 human cases were recorded, mostly men (66.5%), rural residents (67.0%), children under 15 years (49.6%) and more frequent exposure to biting (81.9%). The period 2000-2008 had a higher frequency (85.6%), with 46.6% of cases involving dogs and 45.9% of bats and between 2015 and 2017 three cases occurred for cats (1.6%) with bat variant hematophagous. Median incubation of 50 days and predominant symptomatology was fever (92.6%), agitation (85.2%), paresthesia (66.7%) and dysphagia/paralysis (51.9%). The most frequent states were Maranhão (n=55, 30.0%) and Pará (n=45, 24.0%). The legal Amazon registered 68 cases (36.2%), in rural areas and transmitted by bats. Four cases of human rabies by dogs occurred on the border with Bolivia; the last one in Corumbá/MS, in 2015. Most cases (70.2%) did not perform post-exposure anti-rabies prophylaxis and the others (29.8%) did it inadequately. In the period, 13 patients were treated by the Recife Protocol and two survived. Conclusions: there was a reduction in the incidence of human rabies and a change in the epidemiological profile, predominating cases by bats. It is necessary to investigate possible secondary cases, to enable pre-exposure prophylaxis in populations at greater risk of bite accidents and to strengthen canine vaccination in border areas.

Keywords: Rabies; Rabies Virus; Epidemiology Descriptive; Public Health; Public Health Surveillance.

30th Rabies in the Americas – Kansas City, Missouri, United States of America – October 27 – October 31, 2019

Adherence to guideline recommendations for human rabies immune globulin patient selection, dosing, timing, and anatomical site of administration in rabies postexposure prophylaxis

Presenter: Joshua T. Swan, PharmD, MPH, FCCM, BCPS

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Rabies is a fatal disease that mandates proper prophylaxis after a rabies virus exposure to prevent death. This study evaluated adherence to Centers of Disease Control and Prevention (CDC) recommendations for rabies immune globulin (IG) patient selection, dosing, timing of administration, and anatomical site of administration for rabies postexposure prophylaxis. This retrospective, cross-sectional study included patients who received at least one dose of rabies IG or rabies vaccine at a multi-hospital health system from January 2015 through June 2018. This study included 246 patients, and all of them received at least 1 dose of rabies vaccine. Two patients had a history of rabies vaccination, did not have an indication for rabies IG, and appropriately did not receive additional rabies IG. Rabies IG was administered to 91% (223 of 244) of patients with an indication. Of 223 patients who received rabies IG, 219 (98%) received doses within 10% of 20 IU/kg of body weight, and all 223 (100%) received rabies IG within 7 days of the first rabies vaccine administration. Only 56% (96 of 170) of patients with a wound that could be infiltrated with rabies IG actually received rabies IG via infiltration into and around the wound. This multi-hospital health system study demonstrated high adherence to guideline recommendations for rabies IG patient selection (91%), dosing (98%), and timing (100%). However, only 56% of eligible patients received rabies IG infiltration at wound sites as recommended by guidelines.

Resultados aplicación del modelo pre exposición intradérmico en comunidades indígenas y afrodescendientes de zonas con alta ruralidad de las selva Colombiana 2018.

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Colombia presenta un significativo aumento en rabia humana por animales silvestres, entre 2004 a 2017 ha cobrado 32 víctimas, los departamentos afectados presentan factores de riesgo como zonas de alta ruralidad boscosas y selváticas, alta circulación viral y poblaciones con bajo acceso a servicios de salud e imposibilidad desplazamiento, ausencia de medios de transporte y zonas de conflicto armado, OPS/OMS emitió recomendaciones orientadas a profilaxis preexposición en poblaciones a riesgo, en 2018 recomendó la disminución de dosis en esquemas pre y post exposición, recomendaciones adoptadas por Colombia implementando profilaxis preexposición intradérmica en poblaciones priorizadas (comunidades indígenas – Vichada Cumaribo) y en alerta (Comunidades Afrodescendientes - Antioquia)

Resultados: i).factibilidad: Disponibilidad de 19.966 USD entre la nación y el departamento para el Vichada; para Antioquia de 10.000 USD;la vía intradérmica arrojó una reducción aproximadamente 80% de biológico y sus costos(para vichada se usaron 672 frascos y para Antioquia 193) ii).Cobertura: Se logró la intervención en esquema preexposición intradérmico a 1430 indígenas en Vichada (56 comunidades) y a 969 afrodescendientes en Antioquia(3 comunidades), aplicación intradérmica a toda la población incluyendo niños desde 0 años y gestantes, sin presencia de eventos adversos (primer país de América latina que vacuna el 100% de la población niñez. iii). Adherencia: Para el Vichada, con respecto al 100% de la población que recibió la primera dosis de vacuna se obtuvo una adherencia del 74,1% para la segunda dosis; Antioquia de la población que recibió la primera dosis de vacuna se obtuvo una adherencia del 89%

Rabies Post-exposure Prophylaxis of Veterinarians and Veterinary Staff Following Contact with Rabid Animals, Kansas – 2013-2017

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BACKGROUND: The Advisory Committee on Immunization Practices (ACIP) classifies veterinarians and veterinary staff (VVS) at frequent risk for rabies exposure. Rabies surveillance data was evaluated to characterize VVS rabies exposures and if ACIP postexposure prophylaxis (PEP) recommendations were followed.

METHODS: Rabies-positive animals were entered into Kansas' electronic disease surveillance system and assigned to a local health department (LHD). Standardized rabies risk assessment form, based on ACIP guidelines, was utilized. LHD evaluated all VVS in contact with a rabid animal. PEP recommended based on ACIP exposure risk. From 2013 – 2017 VVS contacts of rabies-positive animals were evaluated for: 1) exposure, 2) PEP recommendation, and 3) PEP compliance.

RESULTS: There were 324/5515 rabies-positive animals; 76% wildlife and 24% domestic. Sixty-one VVS were evaluated; 52 for exposure to large animals (LA) and 9 small animals (SA). PEP recommended for 16/52 VVS exposed to LA; 63% (10/16) non-bite exposures. Eleven VVS considered not exposed and 23 with unknown exposure to rabid LA received PEP. Nine of sixty-one VVS potentially exposed to rabid SA. PEP recommended for 56% (5/9); all non-bite exposures. No additional VVS received PEP due to SA exposure.

CONCLUSIONS: To our knowledge, this is the first evaluation of PEP recommendations and compliance of VVS in the US. Despite ACIP recommendations based on exposure risk, VVS received PEP outside of guidelines. VVS should adhere to veterinary standard precautions to reduce potential non-bite exposures. It is crucial that VVS understand ACIP recommendations to make informed decisions regarding PEP and to provide accurate information to clients with rabid animals.

Treatment of Human Rabies

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POSTER PRESENTATIONS

PP1-Rabies Exposure and Case Tracking Tool for smartphones and tablets

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The Rabies Exposure and Case Tracking Tool (REaCT) is a case management tool designed to aid field investigators conduct rabies surveillance. It was pioneered in Haiti in June 2018, using Integrated Bite Case Management (IBCM). The program was piloted with 11 investigators in five of Haiti's ten departments. REaCT was expanded nationwide in Haiti April 2019. REaCT is designed to improve field level data collection and guide surveillance field staff during investigations. With this application the number of rabies investigations, human exposure and animal assessment for rabies, quality and timeliness of reporting has improved. Since June 2018 REaCT has been used to track over 2500 suspected rabies exposures in Haiti. REaCT allows real-time reporting to national authorities and partners. Algorithms incorporated into the program provide real-time guidance to user, such as the current status of the animal and case out-come (suspect, probable, confirmed, non case). The system is highly adaptable with easy integration of local variables reflective of rabies investigation practices. It is now being used in 5 countries. Highlights of its capabilities, ease of integration ad drawbacks will be presented.

PP02-Fc Engineering of Anti-Rabies Virus Therapeutic Human IgG1 to Alter Binding to the Neonatal Fc Receptor in Nicotiana Tabacum

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Plant expression systems has several advantages such a large-scale production of mAb and absence of pathogenic animal contaminants. However, oligomannose (OM) type glycans structure of mAbpK SO57 in ER have showed a faster clearance compared to antibodies produced in animal cells. The neonatal Fc receptor (FcRn) regulates the persistence of IgG by the FcRn-mediated recycling pathway, which salvages IgG from lysosomal degradation within cells. In this study, Fc-engineering of plant-derived mAb SO57 with an ER-retention motif (KDEL) (mAbpK SO57) was conducted to enhance its binding activity to human FcRn (hFcRn), consequently improve its serum half-life. ELISA and SPR assay showed altered binding affinity between the Fc region of three different mAbpK SO57 mutants [M281Y/S283T/T285E (MST), M457L/N463S (MN), H462K/N463F (HN)] and hFcRn compared to wild type of mAbpK SO57. N-glycan structure was also confirmed that all of mAbpK SO57 mutants had OM type glycans structure similar to the parental mAbpK SO57. In addition, after engineering of mAbpK SO57, the three variants were effective as mAbpK SO57 in neutralizing the activity of the rabies virus CVS-11. This research was supported by a grant (Code#PJ0134372019) from the Korean Rural Development Administration, the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean government (MSIT) (No.2019M3E5D5067214

PP3-Barriers of Attendance Canine Rabies Vaccination in Haiti, 2017

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We conducted a cross-sectional survey to better understand the barriers to attendance at canine rabies vaccination clinics in Haiti. A structured community-based questionnaire was conducted over a 15-day period during May–June 2017, focused on socio-economic status correlated with participation at canine rabies vaccination clinics. Questions phrased as a bidding game were asked to determine individuals' willingness to pay (WTP) for dog rabies vaccination and willingness to walk (WTW) to fixed-point vaccination clinics. The Kaplan-Meier estimator was applied to determine relationships between survey variables. Logistic regression was used to examine factors influencing participants' WTP and WTW. A total of 748 households from eight communities were surveyed. The total number of owned dogs reported from households was 926. The majority of dogs (87.2%) were acquired for security and 49% were allowed to roam freely; 42.0% of dog owners reported that they were unable to walk their dogs on a leash. Seventy percent of dog owners were willing to pay up to 15.9 gourdes (0.25 USD) and/or walk up to 75 meters to vaccinate their dogs. Households that owned free-roaming dogs, owned dogs for the purpose of companionship, and owned dogs which they were unable to walk on a leash were associated with a higher WTP for vaccination. On the other hand, living in Artibonite Department, having a middle or higher household income, and owning a dog for security purpose were associated with a higher WTW for vaccination. Low leash use and propensity for dogs to roam freely are barriers to successful fixed-point vaccination methods in Haiti, and alternative methods should be explored.

PP04-Purification of Plant-Derived Anti-Rabies Virus Monoclonal Antibody Through Optimized pH Conditions for Coupling Between Protein A and Epoxy-Activated Beads

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The main goal of this research was to determine optimum pH conditions for coupling between protein A and epoxy-activated Sepharose beads for purification of monoclonal antibodies (mAbs) expressed in plants. To confirm the effect of pH conditions on purification efficacy, epoxy-activated agarose beads were coupled to protein A under the pH conditions of 8.5, 9.5, 10.5, and 11.5 (8.5R, 9.5R, 10.5R, and 11.5R, respectively). Three hundred grams of fresh leaf tissue of transgenic Arabidopsis expressing human anti-rabies mAb (mAbP) SO57 were harvested to isolate the total soluble protein (TSP). An equal amount of TSP solution was applied to five resin groups including commercial protein A resin (GR) as a positive control. The modified 8.5R, 9.5R, 10.5R, and 11.5R showed delayed elution timing compared to the GR control resin. Nano-drop analysis showed that the total amount of purified mAbPSO57 mAbs from 60 g of fresh leaf mass were not significantly different among 8.5R (400 µg), 9.5R (360 µg), 10.5R (380 µg), and GR (350 µg). The 11.5R (25 µg) had the least mAbPSO57. SDS-PAGE analysis showed that the purity of mAbPSO57 was not significantly different among the five groups. Rapid fluorescent focus inhibition tests (RFFIT) revealed that virus-neutralizing efficacies of purified mAbPSO57 from all the five different resins including the positive control resin were similar. Taken together, both pH 8.5 and 10.5 coupling conditions with high recovery rate should be optimized for purification of mAbPSO57 from transgenic Arabidopsis plant, which will eventually reduce down-stream cost required for mAb production using the plant system. This research was supported by a grant (Code#PJ0134372019) from the Korean Rural Development Administration, the Bio & Medical Technology Development Program of the National Research Foundation (NRF) & funded by the Korean government (MSIT) (No.2019M3E5D5067214)

PP5-Systematic Domestic Dog Vaccination Results in Significant Declines in Rabies Exposures and Human Rabies Deaths

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The rabies virus continues to threaten the lives of thousands people across the world and is responsible for an estimated 60,000 human deaths annually, despite the availability of effective vaccines. Most of these deaths result from the bites of infected domestic dogs. When mass domestic dog vaccination campaigns are implemented consistently and comprehensively to achieve high coverage the incidence of rabies can be effectively controlled. However, oftentimes dog vaccination is limited and the ad hoc culling is undertaken which is both ineffective and counterproductive.

We have been conducting free widespread domestic dog rabies vaccination campaigns in Serengeti District in collaboration with the Serengeti District Livestock Office since 2003. We have further compiled comprehensive rabies incidence through exhaustive contact tracing.

Dog vaccination campaigns have steadily improved over time, in terms of the numbers of villages where campaigns have taken place and the total dogs vaccinated. In 2018 over 25,000 dogs were vaccinated, more than double the number vaccinated in 2012. These campaigns have resulted in a demonstrable decrease in dog rabies incidence and human rabies exposures. Less than 3 rabid dogs and less than one human exposure per month were detected in 2018, compared to over 30 rabid dogs per month and around 20 exposures per month in 2012. In contrast, surveillance data in the surrounding districts in Mara region show that rabies continues to persist in areas where dog vaccination does not take place and poses a major burden of disease. This work demonstrates that domestic dog vaccination is effective at controlling rabies incidence and can have extremely valuable public health impacts.

PP6-Operational Tool for Attention of Canine Rabies Outbreaks in Mexico

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Advances in control of canine rabies in Mexico since 1990 to 2000 represent a historical fact for public health (92% reduction in number of cases), however, in those years the existence of sites in the country where they continued reporting cases, keeping latent the possibility of transmission to the human population. That is why in the decade of the 2000´s a detailed rabies control strategy was designed, it was initially applied in outbreaks in the center of the country before the presentation of 248 cases in the State of Mexico in the period 2000-2006 (no cases currently). This methodology is based on house-to-house visits for rabies vaccination and epidemiological field research. With the commitment to eliminate canine rabies, in 2010 the "Guide for the control of the rabies outbreaks in pets" was published nationwide.

The result of this strategy is the control of the outbreak in Tuxtla Gutiérrez, Chiapas, in 2014 (15 cases from 2011 to 2014), visiting 161,929 homes, with 128 brigadistas (veterinarian) and 13 coordinators for the vaccination of 57,001 animals, with an investment of 4.7 million of pesos (240 thousand dollars) in the period from February to August of that year. After the operation, there were no more cases in such region.

Similarly, in the country this operational tool was applied to the case report in dogs, so that since August 2017 there are no reports of the disease in this animal species.

PP8-Casos de Rabia Paralítica Bovina en el Estado de Nuevo León

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La rabia paralítica bovina es considerada endémica en 25 Estados de la República Mexicana, esto se establece por la presencia del murciélago hematófago, el número de casos positivos y por las condiciones ambientales. En el oeste, este animal se distribuye desde el sur de Sonora hasta Chiapas por la costa del Pacífico y por la costa este, en el Golfo de México desde el sur de Tamaulipas hasta la península de Yucatán (SENASICA). El Estado de Nuevo León es considerado libre de Rabia paralítica bovina, pero en Septiembre del 2018 en el Ejido El Rodeo, Arramberri, N.L.; con una población total de 36 personas conformadas por 11 familias, se presentaron los primeros reportes de ganado con signos clínicos compatibles con rabia paralítica bovina: parálisis del tren posterior, incoordinación, dificultad para deglutir, muerte de 3 a 7 días, se tomaron muestras del ganado que había fallecido y se enviaron a diagnóstico al Laboratorio Estatal de Salud Pública de N.L., confirmando el primer caso positivo de derriengue en el Estado. Al Ejido acudió personal del Departamento de Zoonosis Estatal, personal de la Jurisdicción Sanitaria No. 8, así como personal de la campaña de rabia paralítica bovina. Se han realizado actividades conjuntas en un plan estatal piloto en un radio de 10 km alrededor del foco rábico: se iniciaron 17 esquemas de vacunación en personas que habían estado en contacto con el ganado sospechoso, se aplicaron 685 dosis de vacuna antirrábica en perros (119 hembras – 457 machos) y gatos (43 hembras – 63 machos) y 1200 dosis de vacuna antirrábica en ganado (bovinos, caprinos, ovinos), se tomaron muestras del ganado fallecido logrando confirmar 4 muestras positivas más, se impartieron platicas de concientización a 854 habitantes en los ejidos del Rodeo, Joyas de Bocacelli, Lampacitos, San Rafael de los Cortés, Ibarrilla.

PP9-Estrategias de Vacunacion Antirrábica Canina y Felina en el Estado de Campeche

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La rabia es una enfermedad viral que puede llegar a ser mortal si no es tratada con oportunidad. Según la OMS en el 99% de los casos humanos que ocurren en el mundo, el virus es transmitido por la saliva de perros infectados a través de mordeduras principalmente. Las Semana Nacionales de Vacunación Antirrábica Canina y Felina se inician en México en 1990, son 30 años del desarrollo de esta actividad tanto en el país como en el estado, cada año se realizan dichas jornadas, la principal en el mes de marzo y en septiembre la de reforzamiento.

El presente trabajo es un proyecto descriptivo, de las estrategias operativas y de comunicación Social que se realizan para llevar a cabo la vacunación canina y felina, durante las semanas nacionales de vacunación en el estado de Campeche. Describiendo las estrategias de coordinación intersectorial, planeación, gestión de insumos y biológico, así como de capacitación del personal.

En las campañas de vacunación antirrábica canina y felina que se llevaron a cabo en el Estado de Campeche, en el 2018 se cubrió más del 100% de la meta de vacunación anual (197,500 dosis) como resultado de la coordinación intersectorial de la Secretaria de Salud con IMSS Bienestar, SEDENA, SEMAR, Colegio de Medico Veterinarios, Universidad Autónoma de Campeche, Policía Estatal Preventiva, H. Ayuntamientos de los municipios y personal voluntario; estas estrategias nos han permitido no tener casos de rabia humana desde hace 26 años y canina dese hace 18.

PP10-Pathogenicity Investigation of Taiwan Ferret Badger Rabies Virus on Gem-faced Civets

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E-Mail: Monday October 28, 2019

Since the first case of Taiwan ferret badgers rabies found in 2013, there have been 789 positive animal cases diagnosed till end of August, 2019. The surveillance revealed that Formosan ferret badger has been the major rabies-affected species in Taiwan with positive detection rate of more than 99%. However, there were still spill-over recorded with 6 positives cases in gem-faced civets, 1 positive in a house shrew, and 1 positive in a dog. Considering gem-faced civets had the second-ranking positive detection rate, as well as being the sympatric carnivores with ferret badgers, the pathogenicity of Taiwan ferret badger rabies virus in gem-faced civets was investigated from different aspects. First, five stains of virus harvested from brain/salivary gland homogenates of rabid gem-faced civets were conducted MICLD50 titration, and the result showed the infectious titers were low (in the range of 0 to 1,000 MICLD50). In order to clarify the possibility of cross-infection from ferret badgers to gem-faced civets, the salivary gland homogenate of Taiwan ferret badger rabies virus was inoculated to 2 gem-faced civets in respective group through intramuscular (higher than 106 ferret badger IM-LD50) or intracranial (higher than 100 ferret badger IM-LD50) routes, the results demonstrated all gem-faced civets survived for the observation periods (half year for IM route; two months for IC route). Moreover, one of the IC-inoculated gem-faced civets developed seroconversion. In conclusion, the forgoing results and sporadic cases of gem-faced civets might indicate potentially low cross-species transmission possibility from ferret badgers to gem-faced civets.

PP12-Percepción de las Estrategias de Vacunacion Antirrábica Canina y Felina en Niños del Estado de Campeche

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Dentro de las estrategias para prevenir la Rabia se realizan campañas de vacunación canina y felina, así como la difusión de información para crear conciencia en la comunidad sobre la importancia de la vacunación en estas especies.

El objetivo de esta investigación fue identificar el conocimiento en los niños sobre el estado de vacunación de los perros y gatos con los que habita, así como identificar la convivencia de otras especies con ellos.

El presente trabajo es un proyecto cuantitativo, transversal, observacional, analítico y prospectivo, realizado en los municipios de Hopelchén, Campeche, Champotón y Seybaplaya durante el año 2019, el tamaño de la muestra fue obtenido de forma no probabilística, con un muestreo por conveniencia, se seleccionaron estudiantes de educación primaria de entre 9 y 12 años de edad.

Dentro de los resultados se observó que los niños con perros y gatos referían que el 71% de ellos estaban vacunados y se obtuvo el hallazgo de mascotas silvestre conviviendo en un ambiente domestico sin esquema de vacunación.

La difusión de las campañas de vacunación a través de los diversos medios de comunicación y de la participación intersectorial permitió cumplir con la meta anual de la cobertura de vacunación antirrábica canina y felina, influyendo de forma positiva en los niños, quienes reconocen en todos los municipios el estatus de vacunación de su mascota, infiriendo que a mayor cobertura mayor conocimiento del estado de vacunación de sus mascotas en la población escolar.

PP13-Barriers to rabies post-exposure prophylaxis in the Congo Basin: results from three rabies KAP surveys

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Africa is responsible for 36.4% of the global burden of human rabies deaths. Disease persists due to high rates of dog bites and a lack of established disease surveillance systems, among other factors. Therefore, it is crucial to understand post-exposure healthcare-seeking behaviors to improve post-exposure prophylaxis (PEP) uptake and adherence. Between 2010 and 2013, rabies knowledge, attitudes, and practices (KAP) household surveys were conducted in three countries in the Congo Basin. Findings from Cameroon were published in 2018, Uganda findings are under peer-review, and Democratic Republic of the Congo (DRC) findings are not yet published. This abstract compares findings across these three studies. Data were analyzed for each site using similar logistic regression modelling methods to determine associations between rabies knowledge, dog ownership practices, or demographic characteristics and post-bite healthcare-seeking behaviors. Surveys conducted among 208 households in Cameroon, 798 households in Uganda, and 537 households in DRC found dog-bite rates of 2.6%, 2.3%, and 3.2%, respectively. In Cameroon, increased wealth and knowledge were associated with increased likelihood of seeking medical care and PEP after a dog-bite. In Uganda, higher household poverty was associated with use of traditional medicine after a dog bite. In DRC, higher wealth was associated with increased likelihood of a respondent seeking care, and higher knowledge with receiving PEP, if a dog bite exposure were to occur. Overall, wealth and knowledge play a role in post-exposure decision-making, indicating a need for effective rabies education in the region to increase awareness of the importance of life-saving post-exposure care.

PP14-Puppy pregnancy: the burdensome legacy of the Nerve Tissue Vaccine in India

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With an estimated population of 60 million dogs and 21,000 projected human cases, India is clearly a hotspot for rabies. Several factors are currently hampering its control in the country, including its status as non-notifiable disease and the absence of intersectoral collaboration. Yet, a poor understanding of the sociocultural context of rabies in this unique country also contributes to worsen the problem. In fact, the WHO and the OIE have recently recognized the primary need of considering the sociocultural background as the single most important factor for any rabies control strategy to succeed. Based on my ethnographic research in Delhi slums with rural migrants from Central-Eastern India, I argue that India's prolonged use of NTV – officially discontinued in 2004 – is deeply affecting the effectiveness of PEP through CCV. Coupled with minimal rabies education initiatives, insufficient health services, and inadequate competence attested in some health professionals, the memory of the length and the painfulness of PEP through NTV is still much vivid in the country and it is able not only to discourage people from seeking medical assistance, but also to increase hard-to-dispel rabies-related myths. In fact, I claim that NTV is closely related to the idea, widespread in Central-Eastern India, that dog bites can get a person pregnant with puppies and bound to eventually die from pregnancy or delivery complications. This unique belief and the fear of NTV have to be given appropriate consideration for PEP to succeed in the country.

PP15-Knowledge, attitudes, and practices of dog vaccination, ownership, and health seeking behaviors in peri-urban and urban communities in Bangladesh, 2018

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Bangladesh has the third highest number of human deaths from rabies in the world, about 96% of which result from canine transmission. Dog vaccination against rabies is considered one of the most effective strategies at preventing deaths from rabies, and Bangladesh has declared dog vaccination a key strategy in their plan to eliminate rabies by 2030. A rabies knowledge, attitudes, and practices survey was conducted in 2018 in four vaccination sites representing peri-urban and urban locations in Bangladesh. Data were collected from 2,447 households identifying 86 bite victims and 136 dog-owning households. Responses were used to assess bite rates, rabies knowledge, attitudes regarding rabies, perceptions of dog vaccination and ownership, and healthcare-seeking behaviors among bite victims. The resulting estimated yearly bite rate for Bangladesh is 0.6%. Responses to knowledge based questions indicated the mean knowledge score of participants was 33.1/100 (95% C.I. 31.7, 34.5). The average maximum distance that dog owners are willing to travel to get their dog vaccinated was 3.6 km (95% C.I. 2.8, 4.4). Of 183 owned dogs, 89.7% are allowed to roam freely. Respondents were willing to pay $48.40 USD to receive post-exposure prophylaxis (PEP) with rabies immunoglobulin. Of those who experienced bites within the past year, only 13 (14.9%) reported following treatment guidelines recommended by World Health Organization of washing the wound, seeking medical treatment, and receiving PEP. To reduce the burden of rabies in Bangladesh, gaps in knowledge should be addressed and vaccination strategies must reach owned dogs that are allowed to roam freely.

PP16-Indicators of Rabies Elimination Readiness

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Rabies is a viral zoonosis that causes an estimated 59,000 human deaths annually. It remains endemic in many countries throughout the world, and the World Health Organization (WHO) has set an international goal of eliminating dog-mediated human rabies deaths by 2030. In this study, we model economic, environmental, social, and health determinants to identify the most predictive factors of countries' rabies elimination readiness score (ERS) and to assess countries' readiness to approach rabies elimination. Linear regression was used to assess bivariate and multivariate associations between 24 determinants and countries' ERS, a score based on a country's dog vaccination coverage, availability of post-exposure prophylaxis, and rabies deaths per capita. Backwards elimination was used in multivariate analysis to create a final adjusted model of the most predictive determinants of rabies ERS. Countries' individual data were re-run through the final adjusted model to create a new country score of readiness to approach rabies elimination. Determinants that remained significant in the final model include rate of urbanization, human development index, infant mortality, physician density, and amount of money spent on pets. Scores resulting from running country data through the new model show a difference in predicting rabies elimination readiness than the ERS scores among countries that have not yet eliminated rabies. The scores resulting from this new model show a country's current progress in rabies intervention, and along with rabies ERS, provide insight into which public health factors should be considered when implementing a strategic program to achieve dog-mediated rabies elimination by 2030.

PP17-Rabies Outbreak Investigation — Pedernales, Dominican Republic and Anse-a-Pitre, Haiti, 2019

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Background: During July-December, 2018 three cases of probable rabies in children were reported to Ministerio De Salud Pública (MSP). These were the first cases reported from Pedernales in more than 30 years. All three cases were confirmed at the Centers for Disease Control (CDC). Due to the close association with Anse-a-Pitre, a bi-national coordinated outbreak response was initiated.

Methods: Surveillance data and medical records were reviewed and a household survey was conducted to identify additional cases, detect high-risk bites, and evaluate the size and composition of canine populations in Pedernales and Anse-a-Pitre. Factors contributing to stray dogs, access to medical care and canine vaccination status were evaluated.

Results: Surveys were conducted in 224 and 92 households in Pedernales and in Anse-a-Pitre respectively. Eight (26%) dog bite victims in Pedernales and 3 (27%) in Anse-a-Pitre were reported and having received no medical care. The most commonly reported factor contributing to stray dogs in Pedernales was abandonment of puppies (62.3%) and, in Anse-a-Pitre, people feeding stray dogs (68.2%). Most (87.4%) respondents in Pedernales knew where they could receive rabies vaccination whereas, only 14.6% knew in Anse-a-Pitre. Among dogs owned by surveyed households, 175 (75.8%) and 2 (3%) were reported as having received a rabies vaccine within the past year in Pedernales and Anse-a-Pitre, respectively.

Conclusions: Rabies prevention and control activities including yearly canine vaccination campaigns should be coordinated between Pedernales and Anse-a-Pitre. Education about human and animal rabies and availability of vaccine should be made a priority within both communities.

PP18-Evolución operativa e impacto epidemiológico del Programa de Rabia en Puebla, durante diversos estilos de gobierno

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El registro oficial de casos de rabia en perros, oscilaba entre 300 a 400 por año e incluía los diagnosticados de forma clínica, por laboratorio y los desaparecidos. Evidentemente y con el paso de los años, además de la experiencia acumulada en campo y en gobierno, damos cuenta de que era evidente una falla operativa y tal vez técnica en los conceptos y alcances de un programa estatal, lo que provocaba que murieran por año hasta 15 personas por esta causa

El primer reto fue incluir al Médico Veterinario en el equipo de salud pública, fueron dos administraciones estatales que omitieron esta importancia (10 años) y a la tercera, que de paso debemos agradecerle, aceptó con conciencia técnica y social la intervención de estos profesionales, desde luego anteponiendo el compromiso de trascender entregando cuentas primero a la población y después al gobierno.

Los tiempos de retirar perros, obtener muestras de encéfalos, vacunarlos y esterilizarlos, han tenido etapas interesantes y necesarias, pero no todas favorables, según la percepción social y la política estatal. Hemos cumplido 20 años sin defunciones por rabia y 10 años sin casos de rabia en perros, contamos con 80 Médicos Veterinarios divididos en cuatro tipos de tareas, fomentamos y coadyuvamos la intervención municipal. Hoy podemos ver y estudiar a los perros y su relación con la sociedad para proponer las mejores medidas en favor de mantener ausente la rabia en Puebla. The official record of cases of rabies in dogs, ranged from 300 to 400 per year and included those diagnosed clinically, by laboratory and the disappeared. Obviously and over the years, in addition to the experience accumulated in the field and in government, we realize that an operational and perhaps technical failure was evident in the concepts and scope of a state program, which caused up to 15 people to die per year from this cause

The first challenge was to include the Veterinarian in the public health team, they were two state administrations that omitted this importance (10 years) and the third one, which we must thank in passing, accepted with technical and social awareness the intervention of these professionals, from then putting the commitment to transcend by giving accounts first to the population and then to the government.

The times of withdrawing dogs, obtaining samples of brains, vaccinating and sterilizing them, have had interesting and necessary stages, but not all favorable ones, according to social perception and state policy. We have completed 20 years without deaths due to rabies and 10 years without cases of rabies in dogs, we have 80 Veterinary Doctors divided into four types of tasks, we encourage and assist municipal intervention. Today we can see and study dogs and their relationship with society to propose the best measures in favor of keeping rabies away in Puebla. "

PP19-Selective involvement of vomeronasal organ in rabies vírus infection

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It´s well established that the pathogenicity and pathology of rabies vírus varies according to the variants but the exact mechanism is still not completely known.Female C57/BL6 mice, 4 to 6 weeks old, SPF, were inoculated by intra muscular route with40 LD50 Dog rabies virus (V2) or 40 LD50 hematophagous bat rabies (V3) virus, both of which were wild-type strains. The animals were separated into 2 groups with 8 animals each and euthanized at 5 and 10 days post-inoculation (d.p.i.) for brain collection. Animals in all of the groups were weighed and evaluated daily for the onset of clinical signs of infection. Whole brain was collected immediatelly after death and had their brains tested and confirmed for the presence of RABV by the real-time PCR technique, only then they were sent to the microarray 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®. Gene enrichement, canonical pathways and gene ontology were determined using NIPA. All animals evaluated were in asymptomatic phase and were positive for the presence of the rabies virus in the brain. We observed the downregulation of vomeronasal genes induced by variant 2 variant 2 and not in variant 3 in asymptomatic phase of the disease suggesting the selective involvolvement of vomeronasal receptors in rabies virus infection due to rabies virus variant 2 but not to variant 3.

PP20-Sequence analysis of Rabies viruses collected from 2004 to 2016 in Minnesota, USA

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Rabies is a zoonotic viral encephalitis that is preventable but nearly always fatal once symptoms start. According to the report of 2017 US rabies surveillance, two human rabies cases occurred from exposure to rabid bats. 93, 651 samples were tested for potential rabies exposures, and 4,454 were diagnosed positive. Most of the rabid samples were not sequenced and little information is known on how rabies was transmitted or spread. From 2004 to 2016, the Minnesota Public Health Laboratory had confirmed 517 positive cases and partial sequences of the nucleoprotein (N) gene were generated. Sequence analyses show that rabies viruses in Minnesota divided into the two major variant types, the north central skunk (NCSK) variant (269 cases) and the bat variant (256 cases). The NCSK contained two clades: one clade (174 isolates) is similar to variants found in Canada and in South and North Dakotas; the other clade (67 isolates) is similar to a variant found in Tennessee. The bat rabies variants contained six clades that are associated with specific bat species. Majority (186 isolates) was found to be associated with Eptesicus fuscus. Other bat variant types are Myotis sp (5 isolates), Lasionycteris noctivagans (24 isolates), Perimyotis subflavus (10 isolates), Lasiurus cinereus (11 isolates) and Lasiurus borealis (1 isolates). Over the period, rabid cases of NCSK variants decreased and bat variants increased. For detailed rabies transmission and epidemiological analysis, longer sequences of N gene or whole genome sequences are needed.

PP21-Comparing Methods of Assessing Dog Rabies Vaccination Coverage in Rural and Urban Communities in Tanzania

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Rabies can be eliminated by achieving comprehensive coverage of 70% of domestic dogs during annual mass vaccination campaigns. Estimates of vaccination coverage are, therefore, required to evaluate and manage mass dog vaccination programs; however, there is no specific guidance for the most accurate and efficient methods for estimating coverage in different settings. Here, we compare post-vaccination transects, school-based surveys, and household surveys across 28 districts in southeast Tanzania and Pemba island covering rural, urban, coastal and inland settings, and a range of different livelihoods and religious backgrounds. These approaches were explored in detail in a single district in northwest Tanzania (Serengeti), where their performance was compared with a complete dog population census that also recorded dog vaccination status. Post-vaccination transects involved counting marked (vaccinated) and unmarked (unvaccinated) dogs immediately after campaigns in 2,155 villages (24,721 dogs counted). School-based surveys were administered to 8,587 primary school pupils each representing a unique household, in 119 randomly selected schools approximately 2 months after campaigns. Household surveys were conducted in 160 randomly selected villages (4,488 households) in July/August 2011. Costs to implement these coverage assessments were $12.01, $66.12, and $155.70 per village for post-vaccination transects, school-based, and household surveys, respectively. Simulations were performed to assess the effect of sampling on the precision of coverage estimation. The sampling effort required to obtain reasonably precise estimates of coverage from household surveys is generally very high and probably prohibitively expensive for routine monitoring across large areas, particularly in communities with high human to dog ratios. School-based surveys partially overcame sampling constraints, however, were also costly to obtain reasonably precise estimates of coverage. Post-vaccination transects provided precise and timely estimates of community-level coverage that could be used to troubleshoot the performance of campaigns across large areas. However, transects typically overestimated coverage by around 10%, which therefore needs consideration when evaluating the impacts of campaigns. We discuss the advantages and disadvantages of these different methods and make recommendations for how vaccination campaigns can be better monitored and managed at different stages of rabies control and elimination programs

PP24-Rabies in Canada – 2018

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In 2018, the CFIA laboratories tested 2842 samples for rabies. The majority of samples came from animals that exposed a person (69.3%); all other samples had only domestic animal contact (19.1%) or no documented contact (11.6%). One hundred and eighty-three (6.4%) tested positive, down from 8.9% in 2017. Of these, 75 (41%) were confirmatory tests on wildlife surveillance samples, initially analysed in provincial laboratories. An additional 6 cases positive on immunohistochemistry (IHC) were reported to the CFIA, but were not submitted for confirmatory testing. At the CFIA most samples were analysed by the fluorescent antibody test (n=2835); 4 were tested by IHC, and 7 by quantitative RT-PCR (human samples, all negative). For the first time since 2014, no rabies cases due to the raccoon variant rabies virus (RRABV) were detected in New Brunswick. In Ontario, RRABV case numbers continued to decrease from the original detection in 2015, with only 65 cases in 2018 (119 in 2017). Bats accounted for the highest proportion of cases in 2018 (61=33.9%), followed by raccoons (50= 27.3%), skunks (37= 20.2%), and foxes (20=10.9%). Among domestic animals, rabies was detected in 10 dogs, 2 bovines, 1 goat, and 1 cat, the result of spill-over of skunk-variant virus in western Canada (n=7), fox-variant virus (1 bovine) in southwestern Ontario, and fox-variant virus in northern regions (5 dogs). One spill-over case due to a bat variant virus was detected in a cat in 2018. Although most cases were in wildlife species (168/183, 91.8%), domestic species accounted for 38.5% (1094/2845) of specimens analysed.

PP25-Epidemiological profile of rabies virus circulation in the state of Ceará - Brazil in recent years

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The rabies virus continues to circulate in several animal species in the state of Ceará. The official diagnostic laboratory in the state, LACEN-CE conducts around 600 annual diagnostic exams of samples from 184 municipalities of Ceará. In the last 4 years, we observed a more active surveillance in the forwarding of samples, which results in a positivity index above 10%. Until the early 2000s, urban rabies posed a risk to the state and dog and cat vaccination campaigns were intensified, resulting in excellent vaccination coverage with a significant drop in human cases transmitted by dogs and cats.

In the table below, we can see the results by species of the last years:

Year	Positive	Negative	TOTAL	% Positive
2016	61	364	425	14,35%
2017	102	577	679	15,02%
2018	70	551	621	11,27%
2019	81	386	467	17,34%

Regarding the positive samples, the main species are bats and foxes, representing more than 70% of the positive specimens, according to the table below:

Year	Marmosets		Dogs and cats	Foxes	% foxes	Bats	% Bats
2016	1	0	10	16,39%	38	62,30%	
2017	5	4	21	20,59%	53	51,96%	
2018	12	2	21	30,00%	29	41,43%	
2019	3	3	6	7,41%	65	80,25%	

Active surveillance of wildlife has greatly helped to control the transmission of rabies to domestic animal and humans and the state has been without human death since 2016. The low incidence in dogs and cats, despite their contact with wild species, demonstrates the effectiveness of the annual public campaign strategy. The population should be aware of bats, avoiding contact of animals with bats, since they represent around 50 to 60% of positive diagnoses of the state.

PP26-Update on in vivo efficacy of small molecular weight compounds against rabies virus

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Update on in vivo efficacy of small molecular weight compounds against rabies virus

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Our goal is to find highly-potent, small-molecular weight (< 500 Da) rabies antiviral compounds. After many iterations, we selected compounds of two separate chemotypes that appear to target different steps in host-catalyzed viral assembly, that were highly effective at arresting the release and expression of a recombinant rabies virus (ERA-NLS/GFP) in cell culture. We previously reported PAV-866, lead compound of the first chemotype, was active in cell culture, but failed to show definitive efficacy in mice. Last year we reported PAV-694 and PAV-636, from the second chemotype, also did not have a significant effect on rabies survival in hamsters. Both results were confounded in part due to an apparent marginal antiviral effect of the vehicle DMSO. Now PAV-867, of the second chemotype, was further modified to reduce toxicity, increase bioavailability and brain exposure, as well as identify a non-DMSO vehicle. PAV-867 was given intraperitoneal, once per day, at 0.4 mg/kg in hamsters challenged with a canine rabies virus variant starting either 1 day or 5 days post-infection. Survival for the group that received PAV-867 starting 5 days post-infection approached significance ($p = 0.07$); while, the new vehicle had no effect on rabies survival. Although the in vivo experiments have not achieved the antiviral effect seen in vitro, additional investigations are underway into disruption of viral assembly machinery during antiviral treatment and the amount of drug penetration in the brain. A combination therapy including multiple antiviral chemotypes and possibly new rabies vaccines, will be tested in future studies.

PP28-Case of canine rabies in Fortaleza-Ceará-Brazil after 14 years: Case analysis

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On November 22, 2018, the Zoonoses Surveillance Unit (UVZ) of the municipality of Fortaleza was informed by the Central Laboratory of Public Health of Ceará (LACEN/CE) on a positive dog for rabies in mouse inoculation test, confirmatory examination of the diagnosis of the disease . On November 05, fluorescente antibody test showed the positive sample.

The report is from a dog of aggressive behavior that attacked its owner and 5 other people in the interval of one year between the first and last aggression and all did the vaccine scheme. The owner possessed the animal for more than 5 years, vaccinated annually against rabies, except in 2017, domiciled with a history of fights with cats that entered the backyard of the property. Although there are no reports of any contact with wild animals, there are many trees in the backyard of the residence and in the surrounding area, which can be shelter for bats. It is noteworthy that the animal did not present any classic symptoms for rabies as a change in behavior, Sialorrhea neither ceased to feed or drink water, despite being aggressive, a fact considered normal in its behavior.

The sample was forwarded to the Instituto Pasteur in SP for characterization of the Viral variant. In 10/12/2018 diagnosed the variant of wild canids. It is noteworthy that for several years the typical rabies virus in domestic dogs does not circulate in the state of Ceará. The main variants of rabies circulating in our state are those connected to the Saguis (SOIM), hematophagous and not hematophagous bats and foxes. The UVZ from Fortaleza shortly after knowing the result of FAT performed vaccine blockade actions, as recommended by the National program for rabies control, vaccinating 327 animals (236 dogs and 91 cats).

It draws attention in the case the total absence, by the account of the owner, of contact with the wild canid. The animal was domiciled due to aggressive behaviour. After assaulting the owner, the animal was under observation in UVZ for 20 days, time when no classic symptom of the disease was observed. By behavior, the euthanasia of the animal was indicated when the virus was identified. There was the prospect of diagnosis pointing bat variant, which was not confirmed. There is no report of leaving the animal of the household, nor of any involvement of the dog with another animal species, except for the Cats.

The Municipal surveillance acted promptly in the blockade and investigation and maintains constant monitoring of the area. The case reinforces the annual vaccination strategy and the importance of maintaining constant surveillance and awareness of the risk population.

PP29-Spatial modelling of raccoon rabies vaccine barrier changes between Canada and the US

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Extensive national and international control efforts are undertaken yearly to reduce the endemic area of raccoon rabies virus variant (RRV). Rabies cases can be minimised and prevented at the landscape-scale using oral rabies vaccination (ORV). Therefore, there is a need to assess how much and how often to shift ORV zones to reduce the extent of the endemic rabies zone without risking re-incursion of RRV into previously disease-free areas.

The effectiveness of ORV depends on achieving sufficient immunity in host populations to halt rabies transmission. Host densities vary by habitat type (e.g. forest-agricultural edge versus large patches of contiguous forest), consequently, ORV intensity should scale with host density to achieve sufficient immunity. Here we used a spatially-explicit individual based model, the Ontario Rabies Model, to assess how changes and shifts to an existing ORV zone across varying landscape configurations affect the risk of RRV breach and re-incursion. Controlled-habitat scenarios were tested alongside larger scale landscape simulations to assess the sensitivity of ORV parameters (e.g. shape and frequency of ORV application) to risk of breach.

Our results show how width of the vaccination zone overlap between shifts and frequency of shifts interact across landscape configurations to affect the risk of breach and re-incursion. Specifically, underlying habitat-specific raccoon density and achieved immunity levels are key determinants of barrier breach risk, timing and the resulting spread pathways. These results are relevant for minimizing risk of breach following ORV zone movements and the development of evidence-based strategies for rabies elimination over broad geographic areas.

PP30-Implications of raccoon movement and resource selection for determining optimal baiting designs for oral rabies vaccines in urban and suburban landscapes

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A key question for rabies management efforts targeting free-ranging carnivores is how to effectively distribute oral-rabies vaccine (ORV) baits spatially in order to maximize host exposure to ORV baits. Host habitat preferences and movement behavior are likely important factors in bait exposure rates because they describe how hosts may bias space use towards particular resources on landscapes. Baiting designs that mimic the spatial behavior of animals could increase bait exposure rates, and thus seroconversion rates, if animals exhibit consistent and sufficiently strong patterns of resource selection. To test this hypothesis, we first estimated resource selection and movement behavior from raccoon (Procyon lotor) GPS-location data (N=26) in Burlington, VT, USA. We then predicted raccoon space use on realistic local landscapes (an 81km2 landscape with 30x30m grid cell resolution and six land cover types) using the estimated resource selection values and compared these to alternative patterns of resource selection (more versus less selective). Finally, we applied different spatial baiting designs (i.e., random, data-based – an actual hand-baiting scenario, or resource-based) and bait densities and evaluated exposure rates to ORV across the different combinations of baiting designs and resource selection behavior within a spatially-explicit individual-based modeling framework. The resource-based strategy that mimics raccoon resource selection patterns further increased seroconversion rates relative to the data-based scenario in our simulations, though the amount of advantage depended on the strength of resource selection by raccoons and raccoon densities. We discuss implications of our results for planning optimal ORV baiting designs in suburban and urban landscapes.

PP31-Implementation summary of OIE Twinning Project for Rabies

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Thanks to the strong support from OIE, an OIE twinning Project between Anses-Nancy and AHRI has started to implement since 2018. In this project, four major work packages were designed for the intention to upgrade AHRI, to bolster veterinary service of Taiwan, and finally contribute itself for regional development of rabies management. The four work packages are 1. Improving quality management of rabies diagnosis and serological tests of rabies; 2. Strengthening surveillance capability of AHRI for rabies control; 3. Elucidating pathogenicity of Taiwan ferret badger rabies virus for rabies management; 4. Developing proficiency testing for rabies diagnosis in Asian region, respectively. In addition, in order to spread the OIE initiative, to advertise the core value of this project, and to facilitate the communication and solidarity of the Asian network, a launching meeting with attendees of national reference laboratories from various Asian-Pacific countries was held in October 2018. In this presentation, the achievements of the first-year implementation will be introduced.

PP33-A model of canine rabies that integrates ecological corridors and urban dog subpopulations

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For the past 4 years, health officials have been battling a canine rabies epidemic in Arequipa, Peru. Arequipa is a metropolitan area that has a large population of dogs, most of which are free-roaming, and has few to no other mammalian species able to maintain rabies. Over 150 rabid dogs have been detected during the current outbreak and these cases are spatially associated with dry water channels. In order to understand the effect of the urban landscape on rabies spread in the city dog population, we tracked dogs with Global Positioning System (GPS) collars and mathematically modeled the outbreak. First, we did a spatio-temporal analysis of the GPS collar data of 23 owned free-roaming dogs to identify how dogs move in the dense urban environment. We identified water channels that are dry most of the year as ecological corridors that facilitate linear dog movements over long distances. We then constructed a 2 population SEIR model that captures how rabies can move through the dog population in Arequipa taking into account these water channels. Model parameters were estimated using multi-year community-based surveys conducted in Arequipa and extrapolated from GPS data in conjunction with published literature data. We explored how dogs that extensively use the water channels to move across the city have the potential to perpetuate rabies spread despite vaccination efforts. We also evaluated different control and elimination strategies applied to the different dog subpopulations.

PP34-A Spatial Analysis of Rabies Studies

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The majority of human rabies deaths occur in developing countries, yet much rabies research is focused in developed countries. We aimed to illuminate and visualize this disparity by performing a scoping review of existing rabies literature from 2000 to 2017, and mapping the location of each study area in juxtaposition to the areas where human deaths occur. Author affiliation is also analyzed as many studies focused on developing countries are performed by researchers in developed countries. These comparisons emphasize the need for increased research in regions where rabies is most impactful.

PP35-Seroprevalence of anti-rabies antibodies in wild boars (Sus scrofa) of Brazil

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Wild boars (Sus scrofa) have been classified as invasive exotic species throughout the world, causing damage to native flora, fauna and potential spreading of rabies and other zoonoses. Accordingly, the aim of the study was to assess the seroprevalence of anti-rabies antibodies in wild boar in southern and central-western Brazilian regions. A total of 80 free-range wild boar serum samples were collected between October 2016 and May 2018, with 44 animals from natural and degraded areas of the Atlantic Forest biome of southern (21 at the Vila Velha State Park, 23 around the park) and 36 from degraded areas of the Cerrado biome of central-western Brazil. The modified Rapid Fluorescent Focus Inhibition Test (RFFIT) was used to assess the presence of rabies virus neutralizing antibodies, values ≥0.10 IU/mL being considered positive. As a result, 9/80 (11.3%) of the samples were positive, being 8/21 (38.1%) in Vila Velha State Park and 1/59 (1.7%) in the Central western Brazil, titles ranged from 0.10 to 0.35 IU/mL. In conclusion, wild boars may play an important role in rabies wildlife cycle of neotropical countries, particularly in naturally preserved areas.

PP36-Vaccination of small Indian mongooses (Herpestes auropunctatus) with ONRAB via Ultralite baits – preliminary results

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The small Indian mongoose (Herpestes auropunctatus) is a rabies reservoir on Puerto Rico. Currently no oral rabies vaccine is licensed for use in this species. The Ontario Rabies Vaccine (ONRAB) is a human adenovirus rabies glycoprotein recombinant oral vaccine licensed for use with striped skunks (Mephitis mephitis) in Canada, and under experimental use in the United States since 2011. ORNAB was found to be immunogenic in captive small Indian mongooses when delivered by direct instillation into the oral cavity, but delivery by bait to mongooses has not been attempted. We offered cheese-flavored Ultralite baits containing ~1.8 mL 109.5 TCID50 ONRAB oral rabies vaccine to 18 captive mongooses. Six control mongooses were sham-vaccinated with Ultralite baits containing water. We collected a serum sample at day 0 and day 14 and 30 post vaccination (PV). Rabies virus neutralizing antibodies (RVNA) were quantified using the rapid fluorescent focus inhibition test and titers > 0.1 IU/mL were considered RVNA positive. All study subjects were RVNA negative prior to a 24hr bait offering. All sham-vaccinates and 13/18 vaccinates consumed the bait. By day 14 PV, 7/18 vaccinates were RVNA positive and by day 30 PV, 11/18 were RVNA positive. Two vaccinates that had consumed baits were RVNA negative on days 14 and 30 PV and all sham-vaccinates remained RVNA negative through day 30 PV. We conclude that ONRAB is immunogenic for mongooses by Ultralite bait delivery, though the current bait design may need further optimization for mongooses.

PP37-Standardized habitat-specific small Indian mongoose density estimates: Implications for rabies control

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Mongooses are a rabies reservoir population on several Caribbean islands where they represent a significant public health threat. Past attempts to control mongoose rabies have included depopulation strategies, without success. Rabies management in wildlife now relies principally on vaccination and current research in Puerto Rico is focused on development of an oral rabies vaccination (ORV) program. The design of ORV programs (density and spatial distribution of baits) relies in part on estimates of population density for the target species to ensure adequate bait availability and herd immunity. Although mongooses occupy a variety of habitats in the Caribbean, few studies have used standardized methods to assess habitat-related differences in densities. In this study, we estimated mongoose densities using a capture-mark-recapture protocol conducted on St-Kitts across two seasons and four representative habitats types found throughout the Caribbean: rainforest, dry forest, grassland/pasture and semi-urban areas. The dry forest supported the highest mongoose density (762±64 animals/km2), which was significantly greater than the estimates from the rainforest (248±23 animals/km2), grassland (366±21 animals/km2) and semi-urban (126±39 animals/km2) habitats. There was no difference in habitat-specific densities measured in July-August versus October-November. These results provide essential information on habitat-specific mongoose densities that can inform the design of ORV programs targeting mongooses in the Caribbean. Moreover, we recently completed a depopulation experiment at the dry forest site where ~40% of the mongoose population was removed. Pre- and post-removal population measurements will provide spatiotemporal data to address the relevance of local mongoose depopulation as a rabies control strategy.

PP40-Novel lyssaviruses identified in bats, Taiwan

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Taiwan lost its rabies free status since rabies virus was identified in wild Formosan ferret-badger in 2013. A nationwide rabies surveillance of wildlife was carried out after the rabies outbreak occurred in Taiwan. In the surveillance, bat is one of the targeted animals because bats are natural hosts of most lyssaviruses, and direct fluorescent antibody test and reverse transcriptase polymerase chain reaction were employed in detecting lyssaviruses in bats. Since the surveillance launched in 2013, lyssaviruses were identified in Japanese pipistrelle (Pipistrellus abramus) in Tainan City (TWBLV/TN/2016), Yunlin County (TWBLV/YL/2017), and Yilan County (TWBLV/YiL/2018) in 2016, 2017, 2018, respectively. The concatenated N-P-M-G-L gene sequence of the identified lyssaviruses showed 62.9%–75.3% nucleotide identities to the other 16 species of lyssavirus, suggesting the identified lyssaviruses may be a new species, designated Taiwan bat lyssavirus (TWBLV). The N gene sequence of TWBLV/YiL/2018 revealed 81.2%–81.3% nucleotide identities and 96.2% amino acid identities to TWBLV/TN/2016 and TWBLV/YL/2017. Moreover, a novel lyssavirus was identified in Nyctalus plancyi velutinus in New Taipei City, 2018, and its concatenated coding gene showed 63.2%–76.6% nucleotide identities to the other 16 species of lyssavirus and TWBLV. The genetic distance suggested that the identified lyssavirus in Nyctalus plancyi velutinus may be another new species of lyssavirus, which designated Taiwan bat lyssavirus II. Our surveillance revealed the presence of at least two different lyssaviruses in bat population in Taiwan.

PP41-Double staining rabies infected bat tissue to determine the location of the virus and better understand the overwintering of the virus.

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As the weather changes in the fall and bats return to their hibernacula for the winter, some bats may be infected with rabies when entering torpor. Little is known about rabies virus survival in over wintering bats. Therefore, many questions remain regarding the survival of host and virus.

The focus of this study was to determine if rabies virus was present in the adipose and/or neuronal cells of the interscapular brown adipose tissue (BAT) using double staining techniques. First, we collected various tissues from rabies positive and negative raccoon, cat, cow, gray fox, red fox, bobcat, dog, red bat, hoary bat, silver hair bat, big brown bat and little brown bat. Tissues were stained with FITC conjugated anti-rabies DFA I or III. Depending on the tissue of interest, the secondary Texas Red labeled antibody specific for one of the following; Anti-PGP, Anti-NeuN, and Anti-Adipose Triglyceride Lipase, was applied.

Results showed extensive staining variation among species. In addition, slides showed a great deal of auto-fluorescing, which resulted in every tissue needing a negative control. Considerable effort was required to determine the correct protocol. Once the appropriate protocol was established, rabies positive bats submitted during the fall and spring were necropsied, and cryo- sections were made of the tongue, salivary gland and BAT. Preliminary results demonstrated the presence of rabies virus antigen in nerves innervating the BAT and salivary glands. Additionally, rabies virus antigen was present in salivary gland parenchyma but restricted to the neurons in the BAT.

PP42-Application of commercially available immunochromatographic test kits in wildlife rabies surveillance in Taiwan

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Taiwan had been a rabies-free country since 1961, and the status was changed in July of 2013 when a ferret badger associated rabies virus was discovered. This study collected 2,324 brain samples of wildlife rabies surveillance in Taiwan, composed of 1,580 Formosan ferret badgers (Melogale moschata subaurantiaca), 350 Formosan gem-faced civets (Paguma larvata taivana), 28 crab-eating mongooses (Herpestes urva), 107 Asian house shrews (Suncus murinus), 134 rodents, 57 dogs, 33 cats, and 35 other species. We tested these animal brain tissues with both fluorescent antibody test and one of three brands of immunochromatographic test at the same time, and the results from fluorescent antibody test were considered as standard. Brand A (Bionote, Korea): from July 2013 to June 2017, 1,875 brain tissues were tested with a sensitivity of 99.29% (563/ 567) and a specificity of 99.92% (1,307/ 1,308). Brand B (Green Spring, China): from July 2017 to June 2019, 449 animal brain tissues were tested with a sensitivity of 98.35% (179/ 182) and a specificity of 99.63% (266/ 267). Brand C (Ubio, India): 26 brain tissues were tested with a sensitivity of 0% (0/ 16) and a specificity of 100.00% (10/ 10). There was no rabies-positive specimens can be detected by Brand C. To the best of the author's knowledge, this study is currently the only long-term and large-scale (7 years and more than 2,300 cases) study for commercially available immunochromatographic test kit for rabies viral antigen. Our study revealed that when a laboratory intends to introduce immunochromatographic test as a part of the diagnostic process, a pilot study is required to confirm whether the interested kits can work well.

PP43-EVALUATION OF RABIES LYSSAVIRUS REPLICATION AND CELL GROWTH IN DIFFERENT CONCENTRATIONS OF N2A CELL LINE

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Once the viral isolation in cell culture is an important technique for rabies diagnosis and for animal substitution, the improvement of this technique is essential. This study aimed to evaluate the Rabies lyssavirus replication in the VICC using initial concentrations of 5x105 and 2.5x105 cells/ml and to compare cell growth curves with final concentrations of 1.6x104 and 1x104 cells/ml. Fourteen samples were selected, and two positive controls. For viral replication test, the 96-well plates, with initial concentration of 5x105 and 2.5x105 cells/ml, were observed at intervals of 24, 48, 72 and 96h after inoculation. For the cell growth curve analysis, cells were added in quadruplicate in 96-well plates with final concentrations of 1.1x104 cells/ml and 1.6x104 cells/ml at the same time intervals. Viral replication was observed in all samples from 24h until 96h, for both initial cell concentrations. Interestingly, it was possible to observe the presence of larger foci at the initial concentration of 2.5x105 cells/ml when compared to the concentration 5x105 cells/ml. In the cell growth curve evaluation, at 1.1x104 cells/ml final concentration, the following cell growth rate was observed: 24-48h/219%, 48-72h/14% and 72-96h/17%, while for 1.6x104 cells/ml final concentration: 24-48h/63%, 28-72h/4%, 72-96h/38%. Student's t-test showed a difference in cell growth at 24 and 72h ($p = 0.0031$), but no difference was observed at 48 and 96h. The results suggest that when there are lower concentrations of cells may increase the cell proliferation phase, which can lead to the formation of viral isolation larger foci.

PP45-Livestock rabies in Brazil's central region: Prevention and control actions in years 2017 and 2018

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Livestock rabies transmitted by bats are endemic at Brazil's cerrado biome. This study's goal was to analyse data from livestock rabies control in the State of Goiás, in years 2017 and 2018. In this sense, all the data regarding livestock rabies surveillance performed by Goiás State government animal health agency (AGRODEFESA) in those years were inserted in worksheets (MS Excel) and analyzed statistically (descriptive statistics). In 2017 and 2018 were vaccinated respectively 21.475.185 and 20.614.356 livestock animals. In 2017 were accounted 53 suspect cases of livestock rabies, in wich 31 cases were positive (25 cattle, 03 horses and 03 bats). Still in 2017, 131 vampire bats control actions were performed (capturing bats and shelter monitoring) besides 3.693 rabies surveillance actions in farms. In 2018 were attended 73 suspect cases of livestock rabies, with the confirmation of 32 positive samples (29 cattle and 03 horses). Also took place in 2018, 156 vampire bats control actions and 5.918 rabies surveillance actions in farms. It's important to mention that there are 121 municipalities considered as high risk for rabies in Goiás State, where the vaccination is mandatory on herbivore livestock animals of all ages. In this study were noticed a 37,74% increase on notification of suspected rabies and a 60,25% increase on surveillance actions. It was also observed that the major part of the disease cases occurred in areas considered as low-risk for livestock rabies. This can be due to the fact that the vaccination is not mandatory in those areas.

PP46-Nanopore sequencing to improve rabies surveillance in the United States

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Good surveillance is key to understanding the distribution and spread of disease and is critical to inform control and response measures. The United States currently has one of the best surveillance systems for wildlife rabies, with over 100,000 samples tested each year and ~5,000 rabid animals identified. The majority of rabies virus strain typing is performed using monoclonal antibodies, which can quickly and inexpensively determine the rabies virus variant of a positive sample. However, antigenic variant information lacks the resolution required to examine rabies virus evolution, track rabies outbreaks, identify some translocation events, and investigate suspected host-switching. We have designed a low cost sequence typing method using Oxford Nanopore sequencing. The accuracy and cost of nanopore sequencing was compared to Sanger and Illumina sequencing. Whole genome nanopore sequencing was used to investigate an increase in rabid cats in Florida and a rabies outbreak in captive Vampire bats from Mexico. Nanopore sequencing was able to produce high quality sequences and identify subtle changes in highly related rabies virus isolates. In parallel, nanopore sequencing of the Nucleoprotein and Glycoprotein genes was performed for many rabies virus variants across the United States. These sequences were added to a database of rabies virus sequences to improve rabies surveillance and for use in outbreaks, host switch events, or translocation/importation investigations.

PP48-First report of rabies in Desmodus rotundus in Nuevo León State, Mexico.

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During 1995-2011 in the USA and Canada, bats were the principal source of infection of most rabies human deaths. A human death was confirmed and identified as rabies virus associated with Silver-haired bat Lasionycteris noctivagans. The increase in the geographic distribution of species such as vampire bat (Desmodus rotundus) in the Americas has had a direct impact on human and animal health. Moreover, regional and global change could lead to modifications in vampire bat behavior and distribution that could increase the incidence of human rabies in Latin America and the potential to spread up to the North. Despite our evidence, there is one report (Lee Dana N et al., 2012), on predictive models that consider that the main part of cattle in the USA will be safe from the negative impacts of the oval impact D. rotundus, notwithstanding global warming trends and the results suggest D. rotundus will be limited by temperature seasonality and not expand into the USA through Mexico.

The aim of this study was to identify the species of bats and the lineage of rabies virus circulating in the Nuevo Leon State, since 2018 to 2019. The viruses were isolated from a bat and six bovines. The fluorescent antigen test (FAT), antigenic characterization with a reduced monoclonal panel, RT-PCR and nucleotide sequencing of the semi-variable region of the nucleoprotein gene was performed. Virus isolates were characterized as AgV11.This is the first report in Nuevo Leon that demonstrates the circulation of rabies virus in bats D. rotundus species.

PP49-Development of New Biotinylated Monoclonal Cocktails for Lyssavirus Antigen Detection using Direct Rapid Immunohistochemistry Test (DRIT).

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A rapid and reliable diagnostic test for rabies diagnosis is critical for both patient treatment decisions as well as for basic surveillance and animal rabies control programs. Primary tests for rabies diagnosis rely on the detection of antigens, primarily rabies virus nucleoprotein, in brain tissue. While the direct fluorescent antibody (DFA) test is the gold standard test for rabies diagnosis, it requires specialized equipment such as a fluorescence microscope. The direct rapid immunohistochemistry test (DRIT) is an alternative antigen detection test that does not have these requirements. This test was developed to meet the diagnostic needs of resource-limited laboratories and to allow testing in the field. The DRIT utilizes biotinylated monoclonal or polyclonal antibodies (mAbs/pAbs) followed by detection using streptavidin-horseradish peroxidase (HRP) and a chromogenic substrate for visualization of rabies virus antigen (specific staining) using a light microscope. We have developed two different monoclonal antibody cocktails for detection of Lyssaviruses. Each cocktail is a mixture of two biotinylated murine monoclonal antibodies directed against the rabies virus nucleoprotein (mAbs-N). The first cocktail (CDC-1) has the ability to detect all the rabies virus (RABV) variants that circulate in the United States and major rabies virus variants in the world. A second cocktail (CDC-2) has the ability to recognize non-rabies lyssaviruses. We will present our research and demonstrate the usefulness of our new biotinylated cocktails in the detection of Lyssaviruses by DRIT.

PP50-Sensitive and specific anti-ribonecleoprotein polyclonal horse IgG for rabies virus antigen detection by direct rapid immunohistochemical test

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A novel diagnostic assay, the direct rapid immunohistochemical test (dRIT), has been reported to have a diagnostic sensitivity and specificity equal to the DFA test while offering advantages in cost, time and interpretation. Most previous studies have evaluated the dRIT

using monoclonal antibody cocktails. We aimed to evaluate the anti-ribonucleoprotein polyclonal IgG for Rabies virus (RABV) antigen detection by dRIT. For this, anti-ribonucleoprotein polyclonal horse IgG was purified by ionic exchange chromatography on QAE sephadex A-50 (GE Healthcare) followed by immunoaffinity chromatography column. The IgG obtained was analyzed by 10% SDS-PAGE and its concentration was estimated by methods absorbance at 280 nm. The unlabeled specific IgG preparation was biotinylated using biotin protein labeling kit (Sigma-Aldrich), according to the manufacturer's instructions. The affinity of biotinylated anti-RNP IgG was evaluated by western blot. CNS samples for rabies suspects of different animal species (bovine, cat, dog, equine and bat) was tested by dRIT, being positive (n=22) and negative (n=20), previously analyzed by DFA. As results, the purified IgG contained one electrophoretic pattern compatible with horse IgG. The biotinylated IgG recognized RNP by western blot. The analyses by dRIT revealed that the biotinylated anti-RNP IgG obtained 100% of diagnostic specificity and sensibility for RABV antigen detection. In conclusion, our results demonstrate that the biotinylated anti-RNP polyclonal IgG may be used as a diagnostic reagent in dRIT, with the expectation of cost reduction and expansion surveillance to developing countries. This work represents an important step forward in efforts to diagnosis of rabies.

PP51-Inter-laboratory test for rabies diagnosis: technical evaluation of two rapid kits

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As in previous years, the European Union Reference Laboratory (EURL) for rabies organised in 2018 an Inter-laboratory trial (ILT) on rabies diagnosis. Contrarily to past years, the 2018 ILT did not aim to evaluate the performance of participating laboratories, but the technical performance of new rapid tests. Two lateral Flow Assays (LFA), namely the Anigen® and the CDIATM Rabies Virus Antigen Rapid Test" (commercialized by Bionote and Creative Diagnostics Cie respectively), were evaluated together with the Fluorescent Antibody Test (FAT). One panel of virus samples (including RABV as well as EBLV1a, EBLV-1b, and EBLV2 strains) was sent to participating laboratories to compare results obtained with these different techniques.

The study revealed that the FAT provided a good agreement toward expected results for both negative/positive samples (99.1%). The Anigen® test produced similar results to the FAT, with only one false negative result (0.5%) reported by all participants and a concordance of 100% for all but one sample demonstrating a good inter-laboratory reproducibility of the Anigen® batch. The CDIA™ test produced reproducible results for RABV samples only. However, it hardly detected BBLV, EBLV-2 and EBLV-1b in most laboratories resulting in a moderate inter-laboratory concordance (58.4% to 82.7%) for these lyssaviruses.

The two LFAs provided reliable and reproducible results on all RABV samples (100%) but lead to heterogeneous performances with other lyssaviruses leading to different levels of diagnostic/analytical sensitivity, specificity. The study confirmed that LFAs must be used with caution and that their validation are of upmost importance before any use in laboratories.

PP52-Development and evaluation of a rabies enzyme-linkes immunosorbent assay (elisa) targeting IgM AND IgG In human sera

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Immunity from rabies depends on rabies virus neutralizing antibodies (RVNA) induced after immunization, however the main protective antibody isotype remains unclear. This becomes particularly relevant after the World Health Organization supported elective rabies vaccine regimens that may influence the development of RVNA classes/subclasses, consequently affecting the timing/effectiveness of RVNA IgG levels with ability to infiltrate to the site of the bite/exposure for neutralization of the virus.

This study aimed to develop and validate a rapid and reliable assay for quantifying anti-rabies antibodies IgM/IgG class-switch in human plasma/serum directed against rabies virus glycoproteins based on an indirect ELISA technique.

Immune response was tracked in eleven individuals naïve to rabies licensed vaccine by quantifying serum titers developed at days D0, D7, D14, D21, D28, D42 post-immunization using the reference Rapid Fluorescent Focus Inhibition Test (RFFIT) and ELISA IgM/IgG assays.

RVNA levels quantified in sera (IU/mL) were at D0 (≤ 0.1); D14 (0.6 to 17.5); D21 (1.2 to 23.9); D28 (1.2 to 106.8) and on D42 (2.9 to 84.7). Specific IgM antibody to rabies glycoprotein (EU/mL) peaked from D7 (0.69 to 1.64) to D21 (1.74 to 16.38) post-vaccination. In contrast, IgG antibody (EU/mL) predominated from D28 (3.25 to 19.14) to D42 (5.85 to 20.58) post-vaccination.

These findings show that levels of anti-rabies virus glycoproteins IgM/IgG at D28 appeared to characterize the immune response class-switch.

This validated assay can reliably determine RVNA's potency and monitor IgG/IgM responses strengthening the diagnostic repertoire for making sound decisions about vaccine regimens and processes related to PrEP/PEP.

PP53-Early Rabies Infection Detected in Two Raccoon Cases by rRT-PCR in Pennsylvania

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Background: Rabies is a leading cause of infectious diseases mortality worldwide with an estimated 60,000 deaths annually. While there is no cure, preventive interventions reduce fatality. The Pennsylvania Department of Health Bureau of Laboratories (PABOL) processes approximately 3000 animals for rabies testing annually. Overall positivity rate is 3.6%, with raccoons (40%) as the leading vector followed by cats, foxes, and bats. Although DFA is the gold standard, there is a need to assess molecular methods.

Methods: In 2016, PABOL participated in a PCR project using the pan-lyssavirus rRT-PCR LN34 protocol. During January 2017 - June 2019, PABOL tested over 5000 samples by DFA first and followed by LN34 rRT-PCR.

Results: Among the over 5000 samples tested, two discordant results were found in raccoons tested in 2017 and 2019, respectively. In these two cases, the initial DFA tests were negative for rabies antigen; however, rabies virus RNA was detected by LN34 rRT-PCR. In both cases, the tissues were reprocessed, taking separate samples from different regions of the brain (cerebellum (CB), brain stem (BS) and spinal cord (SC)). Rabies virus RNA and antigen levels were low in all tissues for both samples, with the highest levels in the SC and BS and the lowest levels in the CB, hence suggesting an early rabies infection.

Conclusions: The rRT-PCR demonstrated increased sensitivity in the two raccoon samples. PCR eliminates non-specific and poor-quality monoclonal antibody issues and the expertise needed for DFA.

PP54-EVALUATION OF THE INDIRECT RAPID IMMUNOHISTOCHEMICAL TEST IN THE RABIES LABORATORY DIAGNOSIS AT THE PASTEUR INSTITUTE, SÃO PAULO, BRAZIL.

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The Direct Rapid Immunohistochemical Test (dRIT) has been shown to be promising and recently was recognized by WHO/OIE as a primary test for diagnosis of rabies. Alternatively, the Indirect Rapid Immunohistochemical Test (iRIT) can be used for the same purpose. The aim of this study was to evaluate the iRIT in the Rabies Diagnostic Laboratory from Pasteur Institute, São Paulo, Brazil. Central Nervous System (CNS) samples (n=150) of different animal species (20 cattle, 10 horses, 61 bats, 29 dogs, 21 cats and 9 non-human primates) were tested by iRIT using an anti-RNP IgG polyclonal antibody produced by the Pasteur Institute (in house) and a commercial anti-rabies virus nucleoprotein IgG monoclonal antibody. The results of the iRIT were compared with the gold standard Direct Fluorescent Antibody Test (dFAT). Regardless of the antibodies used (in house and commercial) the results obtained by iRIT were 26 positive and 124 negative samples. Overall agreement between iRIT and dFAT was high (98.6%,kappa= 0.9535), being divergent results in only in two samples. Based upon these reported results, we can conclude that iRIT is a diagnostic tool that can be used to diagnosis rabies routinely, because it presents high concordance with dFAT. In addition, the antibody for rabies produced by the Pasteur Institute presents an excellent efficacy when compared to commercial and in the future it can be distributed for rabies diagnostic network in Brazil, thus contributing to an increase in the rabies epidemiological surveillance.

PP55-Enhanced rabies surveillance in road kill specimens by real-time PCR

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Road kill specimens are an important source of samples for enhanced rabies surveillance in areas where other methods of sample collection may not be practical. However, the quality of road kill specimens is unpredictable and, in many circumstances, unsatisfactory for DRIT or DFA testing due to degraded sample condition. The high sensitivity of molecular diagnostic methods such as real-time polymerase chain reaction (real-time PCR) holds promise for rabies testing when other options are not practical due to poor sample quality. We conducted a preliminary evaluation of the suitability of real-time PCR to detect rabies virus RNA in road kill samples that were deemed unsuitable for testing by DRIT or DFA. Sixty-two road kill specimens were collected from six U.S. states during 2018 and 2019. Photographs, location, temperature, and details of sample condition were collected for each specimen. Specimens included raccoon (n=50), skunk (n=8), fox (n=2), coyote (n=1) and bobcat (n=1). Of the 62 samples tested, 42 (67.7%) were indeterminate, 18 (29.0%) were negative, and 2 (3.2%) were positive. Both positive samples were raccoons (positivity: 4.0%), including one extremely degraded sample. Although the total number of samples tested was low, the positive rate observed here (3.2%) was higher than positive rates observed in previously reported road kill surveillance studies (<1%). This study represents the first time real-time PCR has been used for rabies testing in road kill samples. The ability to detect rabies virus RNA in severely degraded samples suggests that real-time PCR testing of road kill samples could represent a viable method for enhanced rabies surveillance, which ultimately may help better inform decisions for oral rabies vaccination and other wildlife rabies management actions in the U.S. This ongoing study will seek to increase sample size and analyze the effects of environmental conditions on test results.

PP56-Diagnostic sensitivity and specificity of the indirect rapid immunohistochemistry test for "post mortem" rabies diagnosis

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Different techniques have been developed to improve laboratory-based surveillance for rabies, such as rapid immunohistochemical test. The objective of this study was to determine the diagnostic sensitivity and specificity of the indirect rapid immunohistochemistry test (iRIT) for the detection rabies virus antigen utilizing the direct fluorescence antibody test (dFAT) as a reference. So, we tested 103 Central Nervous System (CNS) samples: bovine (44), horses (19), cats (7), dogs (11), bats (18), marmosets (Callithrix jacchus)(2), skunks (Didelphis) (1) and maned wolves (Chrysocyon brachyurus) (1). For each species (excluding bat), 5 anatomic locations in the brains (cortex, cerebellum, hippocampus, brainstem, and spinal cord fragments) were examined by both techniques on glass microscope slides. As primary antibodies, polyclonal rabies virus anti-nucleocapsid-FITC (Instituto Pasteur, São Paulo) were used for dFAT and the mouse anti-rabies virus total protein (Instituto Evandro Chagas, Belém) for iRIT. Of the 103 samples analyzed, 51 were negative and 52 positive by dFAT, so 101 (98.06%) showed divergent results with the iRIT. Two samples (one feline and one equine species) showed divergent results (dFAT negative and iRIT positive). The results of these samples were confirmed by RT-PCR with variant 3 (V3) compatible genetic sequencing. Thus, iRIT presented 100% diagnostic sensitivity and specificity, while in dFAT it was 96.3% sensitivity and 100% specificity. The agreement between the techniques by Kappa test was 0.96. Our results suggest that iRIT has diagnostic efficacy for detecting rabies virus antigen and could contribute to disease surveillance.

PP58-The effect of sample handling on rabies neutralizing antibody stability

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Background and Purpose: As the number of samples tested within a laboratory increases, so does the potential for sample handling variation. To determine the robustness of rabies neutralizing antibodies in human and animal serum, samples were treated to mimic potential deviations in sample handling protocols. Potential deviations were designed to investigate common client inquiries and possible sample conditions experienced during shipping, processing, and setup. Treatments include the duration of serum at temperature greater than refrigerator (room temperature, zero hours to two weeks), the number and duration of Heat Inactivation treatment (i.e., heat inactivation directly from freezer storage, etc.), number of freeze-thaw cycles (zero, four, or six cycles), and the storage duration of sample dilutions in chamber slides before the addition of virus (zero hours to overnight). Method: A panel consisting of 25 or greater human and animal serum samples was created and aliquoted for all control and treatment conditions. Each panel was then treated per one condition as mentioned above. Once all conditions were completed all panels were tested with a control panel for comparison. Stability was assessed by confirming the results were within the acceptable variation of the assay of +/- two-fold of the control panels' results.

PP59-High Throughput Rabies Serology Lab: Fact or Fiction?

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PP60-Permeability Patterns of Fluorescent Isothiocyanate Dextranin Blood-Brain Barrier of Mice Model

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Rabies is considered as a deadly zoonotic disease especially in lower socioeconomic states. The predilection site of rabies virus (RABV) is brain tissue where it develops severe encephalitis predominantly through neuronal dysfunction. The present study has demonstrated the variations in permeability patterns and corresponding Blood Brain Barrier (BBB) damagein experimental rabid lab modelsthrough use of fluorescent isothiocyanatedextrans (FITC-Dextrans)in three different weights (70kDa, 150kDa and 200kDa).The FITC-Dextran has been extensively used in the study of microcirculation and capillary permeability assessment in the field of pharmacokinetics. The rabies infected mice models were selected in this study. These animals were post-immunized through antibodies that could decrease the death ratio of mice by neutralizing the virus particles. In order to observe these changes, the brain tissues of the mice were processed through immunofluorescence. It was revealed that BBB was considerably been crossed by 70kDa and 150kDa molecular weight of FITC-Dextransand the intensity of fluorescent intensity was focused in the cerebral cortex. The antibodies were not enough to neutralize the concurrent RABV to prevent mice death, while the permeability of BBB was efficient at day 5th of post-immunization. The FITC-Dextran is deduced as an important fluorescent tagged drug that can reveal the pathogenicity of central nervous system based viral infectious diseases.

PP61-Rabies lyssavirus isolated from Myotis sp induces an increase expression of iNOS, TNF-α and caspase-3 in the astrocytes

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Some studies have shown differences in the pathogenicity of Rabies lyssavirus (RABV) strains and it depends on virus's ability to replicate, internalize and spread in the CNS. It is known that RABV isolated from Myotis sp had higher replication rate than Eptesicus sp strains. The expression level of RABV Glycoprotein is correlated directly with the high viral replication rate and with the extent of neuronal death. The replication of RABV in neuronal cells induces Caspase 3, iNOS and TNF-α secretion being able trigger to cell death in the CNS. In pathological conditions, astrocytes release molecules that lead to neuron death. Here we evaluated whether the main effectors of apoptosis and necrosis are involved in rabies virus-induced cell death in CNS of mice infected with RABV isolated from Myotis sp and Eptesicus sp. For this, we assessed these molecules expression in astrocytes by double staining immunohistochemistry. Our study showed that the number of astrocytes expressing caspase-3, iNOS and TNF-α was higher in CNS of mice infected with RABV isolated from Myotis sp than in those with Eptesicus sp ($p<0.05$) and positive control (CVS-31) ($p<0.05$), mainly in the hippocampus, midbrain and cerebellum. In conclusion, a higher Caspase-3, iNOS and TNF-α expression by astrocytes could trigger extent of neuronal death and be related to the several symptoms observed in mice infection with Myotis sp strain, such as shaking and paralysis.

PP62-IgG Fab GLYCOSYLATION DIFFERS BETWEEN NEUTRALIZING AND NON-NEUTRALIZING ANTIBODY INDUCED AFTER RABIES VACCINATION

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The neutralizing antibody response against the Rabies virus (RABV) glycoprotein is important for preventing viral infection, but we lack a comprehensive understanding of the mechanisms by which these antibodies can block virus infection. Based on that IgG Fab glycosylation can significantly affect stability, half-life, and binding characteristics of antibodies, we analyzed the level of Fab glycosylation of neutralizing and non-neutralizing IgG antibody induced by pre-exposure prophylaxis for human rabies. IgGs were purified by immunoaffinity chromatography column. Purified IgG was further digested into Fab domains by papain digestion (Pierce Fab preparation kit) and purified from Fc fragments and undigested IgG by gravity flow over protein A resin. Fractions were pooled and analyzed by SDS-PAGE and direct ELISA to assess purity and affinity, respectively. The IgG Fab glycosylation was evaluated by a lectin affinity assay, using: ConA, S.nigra, T.vulgaris, E.cristagally, U. europeaus. Finally, the avidity of Fab from neutralizing and non-neutralizing IgG was analyzed by indirect ELISA using NH4SCN. As results, we observed Fab from neutralizing IgG had higher avidity (ED50=2.0 versus ED50=1.0) and was more glycosylated when compared to non-neutralizing IgG, mainly glycans containing fucose, galactose and sialic acid residues. These findings suggest although poorly understood, the specific patterns of Fab glycosylation appears to be important for antibody neutralizing activity.

PP64-Make it go Viral: Rabies Science Communication

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Rabies continues to be a major global health threat with mortality rates approaching approximately 60,000 deaths annually. Simply put, in the age of Ebola outbreaks, resurgence of vaccine preventable diseases like measles, and raging antimicrobial resistant microbes, rabies is still king but often flies under our microbial radar (especially in the U.S.). It is without a doubt, one of the most diabolical and anxiety inducing words that a physician utters to someone. When one even begins to think that they have been exposed to the virus, it sets off a smart bomb in your central nervous system that spreads like wildfire to your brain much like an actual rabies virus infection. After 25 years of working with this terrifying yet fascinating disease, I can unequivocally state that this panic inducing state in people occurs when faced with the prospect of a rabies exposure or diagnosis. One issue has continued to be apparent with my rabies experiences and that is the low level of health literacy and understanding about rabies by the public. This presentation will illustrate the use of science communication via social media and other media channels to help raise the level of rabies risk competency.

PP66-STABILITY ANALYSIS OF A NEW FREEZE-DRIED RABIES VACCINE AND ITS PROCESS INTERMEDIATES

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Rabies is a viral preventable infectious disease responsible for an estimated 59,000 deaths annually, despite the availability of effective vaccines. Current prophylaxes are essentially 100% effective in preventing human rabies before and after exposure to this virus. Barriers to implementing rabies prophylaxis include among others, vaccine availability and supply, cold chain requirements, product quality, and shelf life. During the vaccine development, the thermal stability of process intermediates and the final product are essential to reach a high-quality vaccine. The present work aims to show the thermal stability at critical steps during the manufacturing process of a chromatographically purified rabies vaccine. Process intermediates and final product stability were measured by determining the Glycoprotein G levels by ELISA, and additionally, NIH potency assays of the Active Pharmaceutical Ingredient (API) and the final product (freeze-dried rabies vaccine), to determine the efficacy at different temperatures. The different intermediates showed good stability during the manufacturing process, with no significative changes in its glycoprotein G content during at least 4 weeks at 5±3°C. The API and the lyophilized vaccine retained its physical, microbiological and immunological properties within the appropriate specified limits: 24 months at 5±3°C, and also in stressing storage conditions: 6 months at 25°C and 30 days at 37°C. In conclusion, these results are essential for the characterization of each stage during the manufacturing process, and the final product retained its efficacy even when stored in stressing conditions. All these data were submitted as part of the dossier submitted for licensing.

PP67-Inactivated Rabies Virus-Vectored Immunocontraceptive Vaccine in a Thermo-Responsive Hydrogel Induces High and Persistent Antibodies against Rabies, but Insufficient Antibodies against Gonadotropin-Releasing Hormone for Contraception

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Rabies is preventable through vaccination, but the need to mount annual canine vaccination campaigns presents major challenges in rabies control and prevention. The development of a rabies vaccine that ensures lifelong immunity and animal population management in one dose could be extremely advantageous. A nonsurgical alternative to spay/neuter is a high priority for animal welfare, but irreversible infertility in one dose has not been achieved. Towards this goal, we developed a rabies virus-vectored immunocontraceptive vaccine ERA-2GnRH, which protected against rabies virus challenge and induced >80% infertility in mice after three doses in a live, liquid-vaccine formulation (Wu et al., 2014). To improve safety and use, we formulated an inactivated vaccine in a thermo-responsive chitosan hydrogel for one-dose delivery and studied the immune responses in mice. The hydrogel did not cause any injection site reactions, and the killed ERA-2GnRH vaccine induced high and persistent rabies virus neutralizing antibodies (rVNA) in mice. The rVNA in the hydrogel group reached an average of 327.40 IU/mL, more than 200 times higher than the liquid vaccine alone. The Gonadotropin-releasing hormone (GnRH) antibodies were also present and lasted longer in the hydrogel group, but did not prevent fertility in mice, reflecting a possible threshold level of GnRH antibodies for contraception. In conclusion, the hydrogel facilitated a high and long-lasting immunity, and ERA-2GnRH is a promising dual vaccine candidate. Future studies will focus on rabies protection in target species and improving the anti-GnRH response.

PP68-Development of a recombinant poxvirus rabies vaccine for bats using a mouse model

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Bats (Order Chiroptera) are the primary reservoir of rabies virus and source of the majority of circulating strains in humans, domestic animals, and wildlife. Additionally, rabies infection in bats can result in significant morbidity and mortality, but currently, no available vaccine exists to protect bats against rabies virus. We had previously developed a recombinant raccoonpox (RCN) vaccine candidate expressing a mosaic glycoprotein gene (MoG) that conferred protection in mice and bats when challenged with rabies virus. We have now developed a second-generation mosaic glycoprotein vaccine (RCN-tPA-MoG) by modifying RCN-MoG through the addition of the tPA secretory signal and the strong PrH5m promoter to enhance protein expression and antibody production in animal models. We tested RCN-MoG and RCN-tPA-MoG in vitro using Western blot analysis and immunofluorescence techniques, which yielded comparable results between both constructs. We are currently conducting a mouse study to evaluate and compare the efficacy of RCN-MoG and RCN-tPA-MoG in mice. A comparison of survival between these two vaccine candidates and immunogenicity, as measured by serological analysis, will be presented. Big brown bats (Eptesicus fuscus) have also been obtained for a vaccine efficacy and challenge trial to compare the two vaccine candidates.

PP69-SYN023 Anti-Rabies MAbs: Preparation for Phase 3 Trials

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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 shown high binding affinities and broad-spectrum neutralization activities. Protection against virus challenges was demonstrated in various animal models. Four Phase 1 and Phase 2 human clinical trials have been conducted in the U.S. and China to evaluate the pharmacokinetics, pharmacodynamics, and safety of the product. Preparation for multi-national Phase 3 trials is in progress. The clinical trials data and global regulatory pathway for registrations will be presented

PP71-Innovative Strategies for Patient Contact Following Mass Bat Exposures

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Rabies in humans is almost 100% fatal, but can be prevented with timely rabies post exposure prophylaxis (RPEP). It's also important not to give RPEP indiscriminately due to side effects, cost and availability. The Kentucky Department for Public Health was called regarding RPEP for girls who woke up to a bat in their cabin during a one week camp. Investigation revealed exposure to three different groups. Each camper was interviewed by phone to determine bat exposure, rabies risk, need for RPEP. Contact information was lacking so innovative strategies were employed to ensure 100% contact.

Each camper was assigned a risk category of "high" (direct bat contact, awoke to the bat, slept on top bunk, deep sleeper, on medications), "medium" (unsure of contact, lower bunk) or "low" (awake, in another room). High or medium risk campers were recommended RPEP.

The camp didn't keep contact information and three different leaders had only partial information. The State Health Operations Center was activated to coordinate the response between state and 16 different Kentucky Counties. University of Kentucky students, epidemiologists, nurses, STD investigators conducted interviews. School-based data bases, social media and Lexus-Nexus background databases were used to gather information.

All 87 campers were contacted, 39 (44.8%) received RPEP. Of those, seven were not recommended RPEP, three refused interviews, six high risk did get RPEP, one medium risk refused RPEP.

Innovative strategies were employed to reach 100% contact using a variety of methods, organizations. Collaboration and using ICS structure was vital in orchestrating our response.

PP72-Challenges of human rabies in Brazil in the 21st century: analysis of the epidemiological profile from 2000 to 2017

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Background: Despite advances in the prevention of human rabies, sporadic cases occur in Brazil, being an important public health problem. Objectives: to describe the epidemiological profile of human rabies in Brazil, 2000-2017. Methodology: a series of cases of human rabies reported 2000-2017, with a spatio-temporal distribution. Results: 188 human cases were recorded, mostly men (66.5%), rural residents (67.0%), children under 15 years (49.6%) and more frequent exposure to biting (81.9%). The period 2000-2008 had a higher frequency (85.6%), with 46.6% of cases involving dogs and 45.9% of bats and between 2015 and 2017 three cases occurred for cats (1.6%) with bat variant hematophagous. Median incubation of 50 days and predominant symptomatology was fever (92.6%), agitation (85.2%), paresthesia (66.7%) and dysphagia/paralysis (51.9%). The most frequent states were Maranhão (n=55, 30.0%) and Pará (n=45, 24.0%). The legal Amazon registered 68 cases (36.2%), in rural areas and transmitted by bats. Four cases of human rabies by dogs occurred on the border with Bolivia; the last one in Corumbá/MS, in 2015. Most cases (70.2%) did not perform post-exposure anti-rabies prophylaxis and the others (29.8%) did it inadequately. In the period, 13 patients were treated by the Recife Protocol and two survived. Conclusions: there was a reduction in the incidence of human rabies and a change in the epidemiological profile, predominating cases by bats. It is necessary to investigate possible secondary cases, to enable pre-exposure prophylaxis in populations at greater risk of bite accidents and to strengthen canine vaccination in border areas.

Keywords: Rabies; Rabies Virus; Epidemiology Descriptive; Public Health; Public Health Surveillance.

PP74-Rabies post exposure treatment vs pre exposure prophylaxis for foreign travels in Sri Lanka

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Sri Lanka attracts thousands of tourists from all over the world.Majority of them come from Europe.Most of them stay two weeks to one month enjoying the sea and hiking in highlands.

Many get bitten by free roaming dogs during their stay.Most of the tourists are unable to trace the dog owners in order to get animal vaccination records and end up seeking post exposure treatment in state owned hospitals.

Some of the patients with minor non bled wounds receive Anti rabies vaccine and many of them require immunoglobulin and vaccine course.Equine Immunoglobulin is widely used in Sri Lanka followed by verocell Vaccine for rabies post exposure treatment.Tourists who never planned to stay in hospital during their dream holidays get hospitalized even for few hours and their travel itineraries get changed with upcoming vaccines.Those who were planning to stay away from city life in a remote location and booked hotels in advance to suit their holidays get totally disturbed .

Travelers who had pre exposure prior to their travel get benefited by spending few minutes at a local hospital for a booster dose of vaccine only and their travel plans stay intact.

Many of the travelers visit during winter time in Europe and most of them had planned their journey at least few months or year ahead.Most of them knew about rabies situation in Asia and many of them were aware about pre exposure treatment and its benefits.But we notice only a very few number of travelers had rabies pre exposure vaccines.

Foreigners pay only the cost of medicine if they are admitted to a state hospital and out patient treatment is free of charge in Sri Lanka.The cost of vaccines is taken by the Ministry of Health in Sri Lanka and patients do not pay as vaccines need no admission.

A course of pre exposure taken at right time when tour was planned could save life, time and money.

PP75-Epidemiology of dog bites: information of patients treated at the Health Centre for Zoonoses Control in Lima, Peru, 2017

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Dog bites lead to injuries and disabilities and may transmit infectious diseases including tetanus and rabies. The history of rabies in the capital Lima together with ongoing urban rabies in southern Peru raise concerns about a possible reintroduction of rabies in Lima. The study objectives were to evaluate epidemiological and clinical characteristics of patients attending the Health Centre of Zoonoses Control (HCZC) in Lima during 2017 because of dog bite incidents and to evaluate the quality of routinely collected information. This was a retrospective observational study describing routine data collected at the HCZC. A total of 2584 patients were treated for animal bite incidents, including 2163 dog bite victims. Most patients came from the most populated districts of Lima, the majority were men (n=1244; 58%) and the median age was 31 years. The lower limbs were the most affected anatomical location (n=1251; 57%). Nearly all patients had treated their lesions immediately and approximately half sought care in other health facilities prior to the HCZC. Many had already started antibiotics (n=864) and anti-inflammatory drugs (n=763). Approximately three quarters were considered to have high-risk exposure for rabies; 1767 (81%) started rabies post-exposure prophylaxis; and according to the patient registration file, only 963 (62%) completed it. This study identified obstacles in routine data collection, and the findings may contribute to more efficient and accurate data collection systems. Regular evaluation of epidemiological and clinical characteristics of dog bite victims may improve patient management and help to prevent the reappearance of human rabies in Lima.

PP76-Characteristics of pediatric and adult cases of putative rabies exposure treated in emergency rooms in the United States, reported in the Centers for Disease Control (CDC) National Hospital Ambulatory Medical Care Survey (NHAMCS) between 2002-2016.

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Post-exposure prophylaxis with rabies vaccine and rabies immune globulin (RIG) following suspected rabies exposure (RPEP) is effective management to prevent rabies, for which there is no treatment. RIG is a critical component of effective RPEP in unvaccinated exposed persons.

There is very limited data on the safety and effectiveness of any RIG product in children. Nevertheless, the evidence gap between the disease burden in children and corresponding pediatric research activity is widest in rabies, among all neglected tropical diseases. Since clinical outcomes are universally fatal if left untreated, in the absence of sufficient high-quality trials, authoritative guidelines routinely extrapolate adult trial data to recommend RIG use in children.

Here, we sought to understand patterns of RIG use in children in the United States from 2002-2016 using publically available sample case data found in the NHAMCS database. 75 representative cases of RIG use were identified, of which 20 (26.67%) were in children under 18 years of age. 12/20 (40%) pediatric cases were in males, compared to 21/55 (61.8%) in adults. RIG was known to be administered on the initial visit in 18/20 (90%) of pediatric cases compared to 41/55 (74.5%) of adult cases.

Children under 18 years of age represent a significant proportion of RIG use in the United States. We present demographic characteristics of pediatric cases compared to adult cases. The significant use of RIG in this population emphasizes the need for additional, high-quality clinical trials in this population to support evidence-based treatment recommendations.

PP77-RETROSPECTIVE STUDY OF HUMAN RABIES CASES CONFIRMED BY LABORATORY DIAGNOSIS AT THE INSTITUTO PASTEUR, SÃO PAULO, BRAZIL, IN THE PERIOD 2003-2016

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The aim of this study was to evaluate the data of human rabies cases diagnosed at Instituto Pasteur from São Paulo, Brazil, during 2003-2016. Data were collected from diagnostic request forms and the computer system data (Inforaiva). The data were analyzed descriptively considering the epidemiological variables. A total of 58 human rabies positive cases were diagnosed, of these 71% were men, 24.5% were children between 0 to 10 years old and 24.5% for adults of 31 a 40 years old. Furthermore, 77.6% of aggressions occurred in the Northeast Region and the Maranhão state presented the highest occurrence (71%). More cases occurred in the rural area (38%) than in the urban area (7%). The most frequent route of transmission was the bite (53.4%) and the most commonly reached places were hands (11%) and feet (11%), presenting in most cases single and deep wounds (20%). The most reported symptoms were hyperthermia (26.6%), followed by paresis/paralysis (24.4%). Bats were involved in 28.8% of cases and there is a prevalence of AgV3 (38%). For the ante mortem diagnosis, PCR presented 72.7% of positivity in hair follicle. Spring was the period with the highest number of cases (34.5%). It is important to highlight the high frequency of uninformed data, ranging from 17.9 to 45.2%. As the number of human rabies cases declined, and a greater number of human rabies transmitted by bats was observed, it suggests a tendency of change in the epidemiological pattern in relation to the animal involved in the transmission.

PP78-Rabies post-exposure prophylaxis in residents of Saint Louis County, MO, 2014 to 2018

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BACKGROUND

Administration of rabies post-exposure prophylaxis (RPEP) is reportable in Missouri.

Although Saint Louis County Department of Public Health (DPH) nurse investigators do not make recommendations regarding RPEP, they interview potentially exposed residents and provide education and data on rabies/RPEP. Because substantial time is devoted to counseling potentially exposed residents, we sought to better understand who in Saint Louis County receives RPEP and examine recent changes over time.

METHODS

This analysis included all Saint Louis County residents who received RPEP in 2014-2018.

RESULTS

In 2014-2018, DPH received reports of 411 people who received RPEP. Annual totals were similar from 2014-2017 (median=63), but increased to 149 in 2018 (157.9% increase over 2017). Overall, 54.5% of RPEP recipients were female; there was no significant annual variation by gender (p=0.32). Across all years, 31.4% of recipients were <17 years, 58.4% were 18-64 years, and 10.2% were 65+ years. Median age differed significantly across years (p=0.04). Overall and for four of the years examined, the highest proportion RPEP was administered to Central county residents and the lowest proportion to Outer North residents. Source of exposure was not reliably collected until 2018, when 76.1% of exposures were to bats, 15.2% to dogs, 5.1% to raccoons, and 3.6% to cats.

Conclusions

The number of Saint Louis County residents receiving RPEP dramatically increased in 2018 and the age distribution has shifted in recent years. Understanding who is potentially exposed to rabies and the source of their exposure can help investigators allocate limited resources.

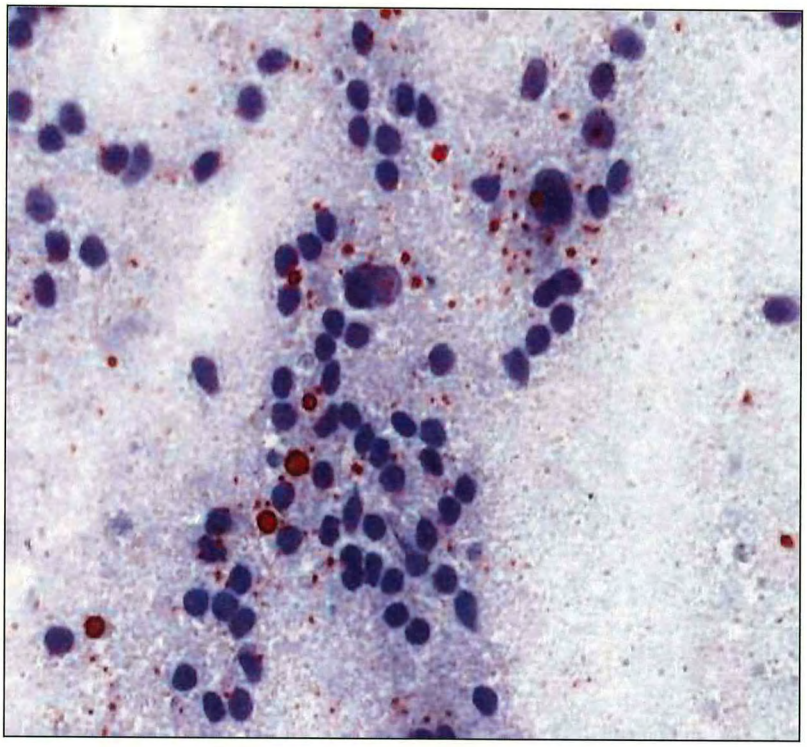
Standard Operating Procedure

For

The Direct Rapid Immunohistochemistry Test (DRIT)

for the detection of rabies virus antigens

October
2019



Contents

1. Introduction

Lyssaviruses, such as Rabies virus, cause an acute progressive encephalitis in warm-blooded hosts, including humans, and the outcome is almost always fatal. Although all species of mammals are susceptible to virus infection, only a few species are important as reservoirs for the disease. Globally, the dog is the most important reservoir. In addition, wildlife taxa are also important. Within North America, several distinct rabies virus variants have been identified in mammalian carnivores, including raccoons, skunks, foxes, and coyotes. In addition to meso-carnivores, multiple species of bats are reservoirs, including representatives in the genera *Eptesicus*, *Lasiurus*, *Myotis*, etc.

Transmission of virus usually begins when infected saliva of a rabid host is passed to an uninfected individual. Various routes of transmission have been documented and include contamination of mucous membranes (i.e., eyes, nose, mouth, etc.), aerosol transmission, and tissue/organ transplantations. The most common mode of virus transmission is via the bite of a rabid animal, allowing virus-containing saliva to enter the victim. Following primary infection, the virus enters an eclipse phase in which it cannot be easily detected within the host. This phase may last for several days, months or even years. The uptake of virus into peripheral nerves is important for progressive infection to occur. After uptake into peripheral nerves, virus is transported to the central nervous system (CNS). Typically, this occurs via sensory and motor nerves at the initial site of infection. The incubation period is the time from exposure to onset of clinical signs of disease. The incubation period may vary from a few days to several years but is typically 1 to 3 months. Dissemination of virus within the CNS is rapid and includes early involvement of limbic system neurons. Active CNS infection is followed by centrifugal spread of virus to peripheral nerves. The amplification of infection within the CNS occurs through cycles of viral replication and cell-to-cell transfer of progeny virus. Centrifugal spread of virus may lead to the invasion of highly innervated sites of various tissues, including the salivary glands, the primary portals for egress. During this period of CNS infection, the classic behavioral changes associated with rabies develop.

The direct fluorescent antibody (FAT) test is the technique used most frequently for rabies diagnosis. The direct rapid immunohistochemistry test (DRIT) is an alternative procedure designed for consideration in rabies diagnostics, according to a standard operating procedure, as recommended for the FAT (e.g., http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.13_RABIES.pdf; http://www.cdc.gov/ncidod/dvrd/rabies/Professional/publications/DFA_diagnosis/DFA_protocol-b.htm). The DRIT may be used to enhance field surveillance of potentially rabid animals, particularly in support of national, regional, state, or local vaccination programs. Recently, the DRIT was recommended by the OIE and CDC as a diagnostic method for the detection of lyssavirus antigens in CNS tissues of rabies suspects.

2. Safety

All persons involved in rabies diagnostic testing should receive pre-exposure vaccination with regular serologic tests and booster immunizations as necessary (e.g., current WHO guidelines; ACIP, MMWR Recomm Rep. 2010 Mar 19;59(RR-2):1-9). Unimmunized individuals should not enter laboratories or areas where such work is conducted. All tissues processed must be disposed appropriately as biomedical waste and all activities related to the handling of samples for rabies diagnosis should be performed using appropriate biosafety practices to avoid direct contact with potentially infected tissues or fluids (e.g., CDC and National Institutes of Health, Biosafety in Microbiological and Biomedical Laboratories, 5th edition, U.S. Government Printing Office). Personnel working with rabid animals and tissues from rabid animals (e.g. CNS) are at risk of virus infection through transdermal injection or contamination of mucous membranes with rabies virus-contaminated material and by exposure to aerosols of virus-infected material. All manipulations of tissues and slides should be conducted in a manner that does not aerosolize liquids or produce airborne particles. Barrier protection is required for safe removal of brain tissue from animals submitted for diagnostic testing. At a minimum, barrier protection during laboratory or field necropsy should include eye protection e.g. safety glasses; face shield; and gloves. When processing multiple animals in a central location, Personal Protective Equipment (PPE) for necropsy may include: appropriate gloves, laboratory gown and waterproof apron, boots, surgical masks, protective sleeves, and a face shield. Fume hoods or biosafety hoods are not required, but they can provide additional protection from odor, ectoparasites, and bone fragments. Besides biosafety concerns, all laboratories should develop a Chemical Hygiene Plan for the proper acquisition, use, storage and disposal of reagents used in the DRIT and attention to use of proper Quality Control methods throughout the procedure.

Poor: Substantial odor, discoloration, liquefaction, desiccation, or an unrecognizable gross anatomy.

Substantial green color, liquefaction, desiccation, or an unrecognizable gross anatomy can indicate an unsatisfactory sample. A substantial loss of tissue during staining and washing or the presence of bacteria on the stained slide may also indicate sample deterioration. If negative results are obtained on deteriorated tissue, the test report should state only that the condition of the sample is such that tests cannot rule out the presence of lyssavirus in the specimen. The negative findings should not be mentioned, since this is often misinterpreted as a negative diagnosis. Positive test results are reported as such. Samples with indeterminate results and all positive samples should be sent for confirmation and typing.

3. Brainstem/cranial cord collection for testing

The critical tissue for rabies diagnosis includes samples from the CNS, in particular the brainstem, which may be obtained from a suspect animal, as follows: Make a ventral midline incision from the symphysis of the mandible to several centimeters caudally beyond the larynx. Sever the musculature and attachments of the tongue on both sides, 3 proceeding caudally to free the larynx, trachea, and esophagus (as if preparing to remove the 'pluck' or tongue, esophagus, trachea, etc., in one piece) and retract to expose the ventral surface of the spinal column and associated musculature. Palpate to identify the atlanto-occipital joint and dissect to expose the connective tissue located on the ventral surface of the joint. This connective tissue is thin and directly overlays cerebrospinal tissue and the spinal cord. With the tip of a scalpel blade, carefully cut through the connective tissue (but not the spinal cord) and work the tip of the scalpel down both sides of the joint, while flexing the joint to gain better access. The exposed brainstem/spinal cord tissue may then be severed as far caudally and rostral as possible to yield CNS tissue suitable for testing. Samples may be placed in screw cap cryovials, preferably unbreakable (i.e. not glass), or other suitable containers, such as ointment tins. Consideration should be given to adequate sample information (such as species, a unique identifying number, date, animal location, etc.). Samples may be refrigerated until testing if they will be tested within a few days. Otherwise, the samples should be frozen, and kept frozen during storage and shipment, until they are tested. To avoid cross contamination of samples, each specimen should be handled on a clean work surface with new disposable gloves. All instruments used during necropsy, dissection, and slide preparation should be decontaminated appropriately and disposable items must be properly discarded as biomedical waste. Instruments not in use should be kept in closed storage. Only those instruments in use for processing a single sample should be exposed. Maintain test samples for ~3 months. Representative positive samples can be subsampled and used as controls. All positive brain samples can be sent for FAT confirmation and variant identification for epidemiologic typing, and for other purposes, along with a sub- sample of negative samples.

Unacceptable deterioration or decomposition of a sample is a qualitative assessment of the condition of each sample upon arrival in the laboratory or at the time of testing. Observation results should be recorded on a DRIT Result Sheet.

Ideal: Optimal brainstem/cranial cord, fresh, no tissue decomposition.

Fair: Slight tissue decomposition but identifiable as brainstem/cranial cord; may have some discoloration around the periphery but stable tissue at the core of the sample.

4. Materials (The suggestion of companies or trade names is for laboratory comparison only and does not imply endorsement)

1. Light Microscope, with 20x and 40x objectives.

2. Tissue-Tek slide staining kit; Fisher Cat. No. NC9479355 (T-Tek #25608902)

3. Slide holders for dipping, 24-place; Fisher Cat. No. NC9418050 (T-Tek #25608868)

4. Syringe 25mm, 0.45 um filter; Fisher Scientific Cat. No. 09-719D.

5. Wheaton glass vials, 8ml; Fisher Scientific Cat. No. 06-408BC.

6. 10cc syringes; Fisher Scientific Cat. No. 14-823-2A.

7. Pipettetor 200ul; Rainin: http://www.rainin.com/ P200 Pipetteman pipette 50-200ul.

8. Corning cover glass, 24x60; VWR Scientific Cat. No. 48396-160.

9. Microscope slides (frosted); Erie Scientific Co. Order No. 10-226.

10. Centrifuge tubes (15 ml), polypropylene; Falcon 352097 Fisher Scientific Cat. No. 14-959-70C.

11. Tips-universal (1 - 200 ul vol); Corning-Costar, Fisher Scientific Cat. No. 07-200-300.

12. Pipette Bulb (0.05 - 100 ml); VWR Scientific Cat. No. 53497-055.

13. Serological pipettes (1.0 ml); Pyrex Corning 7078D-1, VWR Scientific Cat. No. 53222-259.

14. Serological pipettes (5.0 ml); Pyrex Corning 7078D-5, VWR Scientific Cat. No. 53222-281.

15. Serological pipettes (10.0 ml); Plastic, VWR Scientific Cat. No. 20171-042.

16. Tissue culture plate lids (50/case); BD Falcon, Fisher Scientific, Cat. No. 08-772-2B.

Suggested Reagents

*Note: All reagents can be stored at room temperature, unless listed otherwise.

1. Formalin, 10% buffered; Fisher Brand, Order no. SF 100-4 (4L).

2. Phosphate buffered saline (PBS); Fisher Scientific Cat. No. SH30256.02.

3. Hydrogen peroxide, 3%. Use any commercially available, 3% hydrogen peroxide e.g. Pharmacy or Department Store brands, etc.

4. *Primary antibody: Polyclonal anti-nucleoprotein or cocktail of anti-lyssavirus biotinylated antibodies, stored at 4°C. Supplied ready-to-use or as determined by user.

5. *Streptavidin-Peroxidase: 50mL (71-00-38); Kirkegaard & Perry Laboratories Inc, ready-to-use, store at 4°C.

6. Amino-ethylcarbizole (AEC) substrate; SIGMA no. A6926.

7. N,N, Dimethyl formamide; GR, EM Science, Cherry Hill, NJ. Thomas Scientific Order No. C279-A87.

8. Acetate Buffer, 0.1M, pH 5.2; Polyscientific, Bay Shore, NY, (516) 586-0400. Catalog No. S140.

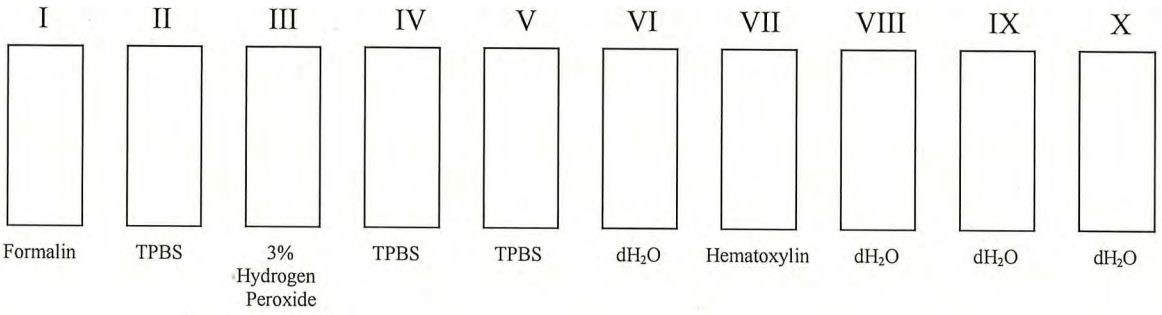
9. Gills hematoxylin formulation #2; Fisher order no. CS401-4D, 4L. Diluted 1:2 in distilled water.

10. Gel/Mount; BioMeda Corp., Foster City CA, (aqueous/dry mounting) Fisher Scientific Cat. No. BM-MO1, 20ml.

11. TWEEN 80, Polyethylene glycol; Sigma-Aldrich order no. P1754.

12. Deionized water; Fisher Scientific, Cat. No. S75232

5. Example of Tissue-Tek staining tray set-up and reagents in staining dishes.



Staining dish number:

I. Formalin - change out after 2 runs or ~once a week.

II. TPBS - change out with each test.

III. 3% Hydrogen peroxide - change out with each test.

IV. TPBS - change out with each test.

V. TPBS - change out with each test.

VI. Deionized/distilled water (dH_2O) - change out with each test.

VII. Hematoxylin - change out once a week.

VIII. dH_2O - change out with each test.

IX. dH_2O - change out with each test.

X. dH_2O - change out with each test.

6. Preparation of reagents for staining dish

I. Formalin, 10% buffered; ready-to-use

II. Phosphate buffered saline with 1% tween-80 (TPBS)

TPBS (PBS with 1% tween-80) (e.g., to 990 ml of PBS add 10 ml Tween-80. Shake until tween-80 is completely into solution)

III. 3% hydrogen peroxide; ready-to-use

IV. TPBS

V. TPBS

VI. Deionized/distilled water (dH_2O); ready-to-use

VII. Hematoxylin

Gills formulation #2 diluted 1:2 in distilled water. The staining dish will hold ~250 ml of solution (125 ml hematoxylin + 125 ml deionized water)

VIII. Deionized/distilled water (dH_2O)

IX. Deionized/distilled water (dH_2O)

X. Deionized/distilled water (dH_2O)

7. Protocol for the Direct Rapid Immunohistochemical Test (DRIT)

A streptavidin-biotin peroxidase staining technique for diagnosis of lyssavirus infection in CNS tissues.

1. Make routine touch impressions of suspect CNS tissues (e.g., brainstem) on labeled glass microscope slides (always include standard positive and negative controls).

2. Air-dry slides for ~ 5 minutes at room temperature.

3. Immerse slides in 10% buffered formalin at room temperature for 10 minutes. **Dish I.**

4. Remove and dip-rinse slides several times to wash off any excess fixative in wash buffer TPBS (PBS with 1% tween 80). **Dish II.**

5. Immerse slides in 3% hydrogen peroxide for 10 minutes. **Dish III.**

6. Remove excess hydrogen peroxide by dip-rinsing slides in TPBS, **Dish IV**. Transfer slides to the next rinse **Dish V** (after dipping, shake off excess buffer, and blot excess buffer from slide edges surrounding the impression). Work with one slide at a time leaving the remaining slides immersed within the TPBS rinse.

7. Incubate slides in a 'humidity chamber' (e.g. may use the plastic top to a 96-well plate or another simple cover over slides, on a moistened paper towel, on lab bench top) at room temperature with primary antibody – e.g. biotinylated anti-rabies poly- or monoclonal antibodies for 10 minutes (add enough of this primary antibody by drop to cover the impression).

8. After incubation shake off excess conjugate. Dip-rinse slides with TPBS, Dish V (shake off excess TPBS and blot buffer from slide edges surrounding the impression). Can use this same wash buffer through step 10.

9. Incubate slides with streptavidin-peroxidase complex (add enough of this complex to the slide by drop to cover the impression) in a humidity chamber at room temperature for 10 minutes. After incubation, shake off excess.

10. Dip-rinse slides with TPBS, **Dish V** (shake off excess buffer and blot excess buffer from slide edges surrounding the impression).

11. Incubate slides with peroxidase substrate, e.g., amino-ethylcarbizole (AEC) – prepare the working dilution (p. 13) just prior to use. Add enough of this substrate to the slide by drop to cover the impression in a humidity chamber at room temperature for 10 minutes. After incubation, shake off excess substrate.

12. Dip-rinse slides in deionized/distilled water, **Dish VI**.

13. Counterstain with Gills Hematoxylin (diluted 1:2 with deionized/distilled water) for 2 minutes, **Dish VII**.

14. Immediately dip-rinse this stain from slides with deionized/distilled water **Dish VIII**. Make a second dip-rinse of slides with fresh deionized/distilled water (**Dish IX**) to ensure removal of excess stain.

15. Transfer slides to fresh distilled water **Dish X**. Shake off water and mount slides with water-soluble mounting medium and cover-slip (work with one slide at a time, shake off excess deionized/distilled water and blot excess from slide edges surrounding the impression). Do not allow slides to air-dry prior to cover-slipping. If multiple slides are stained, they may sit in the deionized/distilled water rinse before cover-slipping.

16. View slides by light microscopy, using a 20x objective to scan the field thoroughly, and a 40x objective for higher power inspection (rabies virus antigens appear as reddish inclusions against the blue neuronal background).

17. Record results (sample #, species, date, locality, drit score, etc.).

8. Preparation of peroxidase substrate: amino-ethylcarbizole (AEC), Step 11, page 12.

A. STOCK solution:

Reagents:
1. Amino-ethylcarbizole (AEC) substrate (e.g.,, SIGMA no. A6926)
2. N,N, Dimethyl formamide GR, EM Science

Supplies:
1. 5 ml Pyrex (glass) pipette
2. 8 ml Wheaton jar

To prepare AEC stock solution

a. Dissolve one 20mg tablet of 3-amino 9-ethyl carbazole (AEC) in 5 ml of N,N, dimethylformamide in a glass Wheaton jar (label 'AEC stock' and date), wrapped in foil.

The AEC stock solution should be stored in the dark in a refrigerator (4 °C) for 1 to 2 months.

B. WORKING dilution; prepare fresh with each test just prior to staining slides:

Reagents:
1. Acetate buffer, 0.1M, pH 5.2
2. AEC Stock (above)
3. 3% Hydrogen peroxide

Supplies:
1. 1 ml Pyrex (glass) pipette
2. 10 ml plastic pipette
3. Pipettetor (200 ul)
4. Pipette tips (200 ul)
5. 0.45 um syringe filter
6. 10 cc syringe
7. 15 ml centrifuge tube (2)

To prepare AEC working dilution:

a. Add 7 ml of acetate buffer to a 15 ml centrifuge tube using a 10 ml plastic pipette.
b. Add 0.5 ml of AEC stock solution (above) using a 1 ml Pyrex (glass) pipette.
c. Add 0.075 ml (75 ul) of 3% hydrogen peroxide.

Filter mixture using a 10 ml syringe with syringe filter (0.45 um) into a separate 15 ml centrifuge tube

This mixture once made is only stable for 2-3 hours, so should be made just prior to use during the staining procedure.

9. Reading and recording results

A test sample can be considered negative for lyssavirus antigens when brain stem/cord is scanned over approximately 20-40 fields at a magnification of approximately 200X or greater for detection of typical viral inclusions.

Test Results

Staining intensity / antigen distribution. Lyssavirus in the brains of infected animals produces intracytoplasmic inclusions of various shapes (see Figures B, C, and D). Negative samples do not have such specific inclusions (see Figure A). A single microscopic field may contain numerous round or oval masses and strings. When stained specifically with biotinylated antibody, the substrate 3-amino-9-ethylcarbazole (AEC), upon oxidation, forms a rose-red or magenta end product. Hematoxylin counterstain will produce a blue color to tissue and nuclear background. The AEC is susceptible to deterioration in excessive light and will fade in intensity. Storage in the dark is therefore recommended.

Observations made for each test slide are recorded as staining intensity/antigen distribution on the data result sheet.

Staining intensity is graded from +4 to +1. Positive control slides in all tests should optimally contain staining of ~+4 intensity. Slightly diminished staining intensity (a slight loss of color) is graded as +3 intensity and may occur in test samples positive for viral antigens when sample handling has not been optimal. Noticeably dull staining is graded +2 to +1 and cannot be considered as diagnostic for a lyssavirus infection without confirmation of specificity. Diminished staining intensity may be the result of denaturation of viral antigens, but less than optimal staining may also result from non-specific binding of antibody to components of inflamed tissue or artifacts of tissue decomposition.

Antigen distribution. For each brain examined, staining is graded by the amount of antigens present as follows:

+4, a large infiltration of large and small inclusions of varying shape in almost every area of the impression.

+3, inclusions of varying size and shape are found in almost every microscopic field, the number of inclusions per field varies, but inclusions are numerous in most fields.

+2, inclusions of varying size and shape are present in 10% to 50% of the microscopic fields and most fields contain only a few inclusions.

+1, inclusions of varying size and shape are present in <10% of the microscope fields and only a few inclusions are found per field (usually only one or two inclusions per field).

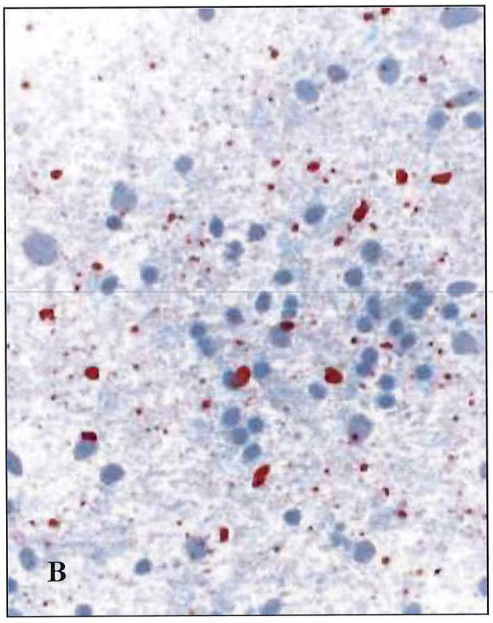
Test interpretation. If the tissue sample was adequate and suitable for rabies diagnosis, results for a test animal are reported as positive or negative for rabies (test complete), or non-diagnostic (test indeterminate) based on observed patterns of staining in test and control slides.

Test complete / reportable result. Test results are reported if the following observations are made:

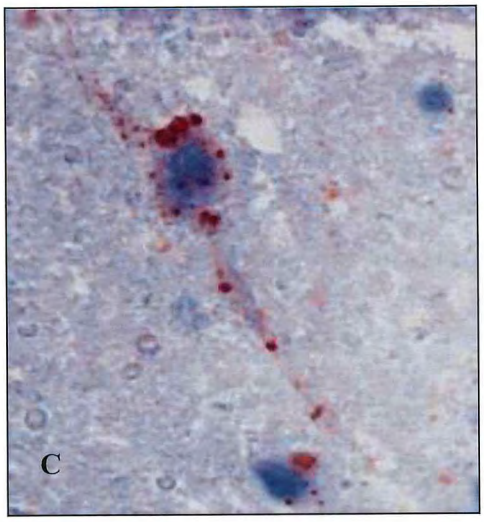
Test controls: Both large and small antigen accumulations in positive control slide stain with +4 intensity and +3 to +4 antigen distribution. No staining is present on negative control slide.

Test samples: No tissue deterioration or alteration was noted when slides were prepared. Samples are clearly negative (no specific staining in test slides) or clearly positive (at least +3 to +4 intensity and +3 to +4 distribution of antigen in slides made from brain stem and/or spinal cord.

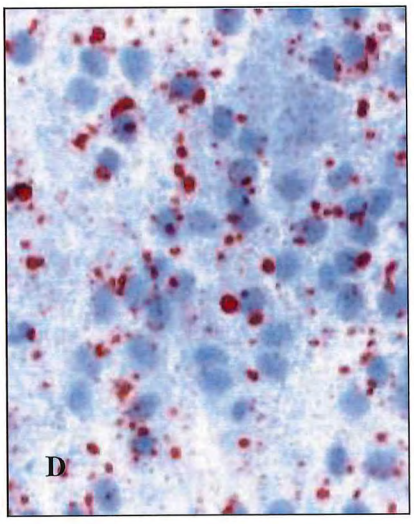
A. Negative brain touch impression



B. Positive reaction (detection of lyssavirus nucleoprotein).



C. Observation of cell-associated intracytoplasmic inclusions characteristic of lyssavirus infection.



D. Positive reaction (400x total Magnification).

Figures A-D courtesy of Michael Niezgoda, CDC, Atlanta, GA, USA.

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