



XXIX INTERNATIONAL CONFERENCE ON RABIES IN THE AMERICAS

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Rita Rabies In The Americas
2018
XXIX

**BUENOS
AIRES
ARGENTINA**



¡Bienvenidos!

Estimados colegas, en nombre del Comité Organizador Local, les damos la bienvenida a la 29a Conferencia Internacional de Rabia en las Américas (RITA).

Esta es la primera vez que RITA se realiza en Argentina y Fundación Pablo Cassará ha tenido el honor de ser responsable de su organización. La Fundación tiene una larga historia de apoyo a actividades científicas y ha contado con la colaboración de colegas que pertenecen a instituciones argentinas con una larga trayectoria en el ámbito de la rabia. Nuestro profundo agradecimiento por su apoyo, compromiso y dedicación.

Las reuniones de RITA son una herramienta fundamental que permite presentar los resultados de las últimas investigaciones en el área, fomentando el análisis y la discusión de los mismos, afianzando lazos personales y científicos que luego redundan en mayores colaboraciones y mejores interacciones.

Esperamos que la sociedad en su conjunto se vea beneficiada por la energía y pasión puesta por investigadores, profesionales de la salud, gerentes internacionales, nacionales, provinciales y locales de programas de rabia, biólogos de vida silvestre y personal de laboratorio que participan en programas de investigación, vigilancia, prevención y control de la rabia. En esta edición nos proponemos mostrar aspectos de la problemática de la rabia en Sudamérica sin dejar de lado a otras regiones, esperando lograr el desafío de alcanzar un balance justo dado que contamos con la presencia de representantes de 22 países de diferentes continentes.

Como presidente del Comité Organizador Local deseo agradecer de manera sincera a nuestros conferencistas, así como a quienes realizarán presentaciones orales o en posters. Gracias a ellos podemos enriquecernos con nuevos resultados y renovadas perspectivas de análisis. Agradezco también a nuestros sponsors que hacen posible que este evento sea un ámbito que genere un impacto positivo en todos los participantes.

Esperamos que se sientan cómodos con la sede elegida, con nuestra ciudad y nuestra gente. A su vez esperamos que en cada uno de nosotros continúe germinando la semilla de la incomodidad y la mejora continua, esa que nos impulsa a enfrentar una realidad que nos duele, la realidad que nos muestra una enfermedad no controlada en muchas regiones y los impactos que esto generan a nivel social y en el desarrollo humano. Gracias a esa incomodidad que no se traduce en una queja, sino en nuestro arduo trabajo y en la elaboración de propuestas hoy nos encontramos compartiendo nuestra profunda voluntad de colaborar con acciones que nos permitan transformar el mundo.

¡Sigamos sintiéndonos inconformes hasta lograr la satisfacción de transformar la realidad!

Dr. Alejandro Daniel Parola

Presidente del Comité Organizador Local
XXIX Conferencia Internacional de Rabia en las Américas (RITA).
Fundación Pablo Cassará

Welcome!

Dear colleagues, in the name of the Local Organizing Committee, you are welcomed to the 29th International Conference of Rabies in the Americas (RITA)

It is the first time that RITA will be held in Argentina and Pablo Cassará Foundation has been honored with the organization of the event. This Foundation is known for having a remarkable track in supporting scientific activities; it has been contributed by colleagues who belong to local institutions with wide experience in rabies studies. Our deepest gratefulness for their support, commitment and dedication.

Meetings at RITA represent a basic tool through which the latest research results in the arena are socialized, promoting their analysis and discussion, reinforcing personal and scientific bonds that impact in greater contributions and better interactions. We hope the entire society can be benefited by the energy and passion shared by researchers, health professionals, international, national and local managers of rabies programs, wildlife biologists and laboratory staff that participate in rabies surveillance, prevention and control programs. In this edition, we have the purpose to show different aspects of rabies in South America considering the other regions as well, hoping to beat off the challenge of reaching a just balance, since we count on the attendance of representatives from 22 countries from different continents.

As the president of the Local Organizing Committee I sincerely thank all our lecturers as well as participants giving their oral presentations or with posters. Thanks to all of them, we can enrich ourselves with new results and fresh perspectives of analysis. I also want to thank our sponsors: they allow our event to be an environment which generates a positive impact on every participant.

We hope you feel comfortable with the chosen venue, with our city and our people. At the same time, we hope the seed of discomfort and desire for improvement keeps on growing in all of you, that seed that encourages us to face a hurting reality, a reality that shows us an uncontrolled disease in many territories and its impacts in social life and human development. Thanks to that feeling of discomfort, which is not translated into complaints but in hard work and elaboration of proposals, we found ourselves sharing our deep will of collaborating with actions that let us change the world.

Let's keep on feeling uncomfortable until we accomplish the satisfaction of transforming reality!

Dr. Alejandro Daniel Parola

President of the Local Organizing Committee
XXIX International Conference of Rabies in the Americas (RITA)
Pablo Cassará Foundation

Bem-vindos!

Prezados colegas, em nome do Comitê Organizador Local, damos a bem-vinda à 29ª Conferência Internacional de Raiva nas Américas (RITA).

Pela primeira vez RITA se realiza na Argentina e Fundação Pablo Cassará tem a honra de ser o responsável pela sua organização. A fundação possui uma longa trajetória de apoio a atividades científicas e conta com a colaboração de colegas que pertencem a instituições argentinas com uma ampla trajetória no âmbito da raiva. Nosso profundo agradecimento pelo seu apoio, pelo seu compromisso e pela sua dedicação.

As reuniões da RITA são uma ferramenta fundamental que permitem apresentar os resultados das últimas pesquisas da área, contribuindo à análise e à discussão dos mesmos, afiançando laços pessoais e científicos que depois redundam em maiores colaborações e melhores interações. Esperamos que a sociedade no seu todo se veja beneficiada pela energia e paixão dos pesquisadores, profissionais da saúde, gerentes internacionais, nacionais, estaduais e locais de programas de raiva, biólogos de vida silvestre e pessoal de laboratório que participam em programas de pesquisa, vigilância, prevenção e controle da raiva. Nesta edição propomos mostrar aspectos da problemática da raiva na Sul-América sem deixar de lado outras regiões, esperando lograr o desafio de atingir um balanço justo já que contamos com a presença de representantes de 22 países de diferentes continentes.

Como presidente do Comitê Organizador Local desejo agradecer de maneira sincera aos nossos conferencistas assim como aqueles que realizarão apresentações orais ou pôsteres. Graças a eles podemos nos enriquecer com novos resultados e renovadas perspectivas de análise. Agradeço também a nossos patrocinadores que fazem possível que este evento seja um âmbito que gere um impacto positivo em todos os participantes.

Esperamos que se sintam à vontade com a sede escolhida, com nossa cidade e com nossa gente. Por sua vez, esperamos que em cada um de nós continue geminando a semente da incomodidade e da melhoria continua, aquela que nos impulsiona a enfrentar uma realidade que nos dói, aquela realidade que nos mostra que uma doença não controlada em muitas regiões e os impactos que isto gera a nível social e no desenvolvimento humano. Graças a essa incomodidade que não se traduz em uma reclamação, mas em nosso árduo trabalho e na elaboração de propostas hoje nos encontramos compartilhando nossa profunda vontade de colaborar com ações que nos permitam transformar o mundo.

Continuemos nos sentindo inconformados até atingir a satisfação de transformar a realidade!

Dr. Alejandro Daniel Parola

Presidente do Comitê Organizador Local
XXIX Conferência Internacional de Raiva nas Américas (RITA).
Fundação Pablo Cassará

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EXPERIENCE OF THE MINISTRY OF HEALTH AND SOCIAL PROTECTION IN THE IMPLEMENTATION OF PILOTING A PRE-EXPOSURE RABIES SCHEME IN INDIGENOUS COMMUNITIES (SIKUANI AND PIAPOCO) VICHADA, COLOMBIA 2016 – 2017

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Presenter: Angela Xiomara Castro Duran

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As the presentation of human rabies cases transmitted by dogs has decreased in Colombia, increase in wild rabies cases in several regions of the country is been seen, among them Vichada. In response, a pilot pre-exposure rabies scheme application was implemented in indigenous populations living in disperse rural areas with limited access to health services and expose to assaults by blood-sucking bats, in the Vichada and Uva rivers of the municipality of Cumaribo - Vichada during the years 2016 and 2017. Results: i). Factibility: It was feasible to apply a pre-exposure rabies schemes to communities at risk with an investment of 76,388 USD. The cost of applying the scheme per dose was 7.8 USD, with the acceptability of the indigenous communities, availability of transportation and adequate human resources in the area. There were geographical access barriers in the summer season; ii). Coverage: The pre-exposure scheme was completed in 2,906 people (71.5% of the total population belonging to 127 communities at risk); A total of 10,206 doses of vaccine were applied without adverse events. iii). Adherence: Of 1,196 people who received the first dose in 2016, 82.5% and 78.9% adhered to the scheme in the second and third doses, respectively; of 2,066 people who received the first dose in 2017, 77.1% and 64.4% adhered to the second and third doses. Conclusions: I). There is financial feasibility, logistics and human resources to develop this type of interventions with adequate coverage and adherence, that can be improved by recognizing the cosmogony and worldview of indigenous people; II). The active participation of the governors of each community is fundamental for the development of this type of activities. III). Of 4,062 people who received the first dose, 79.7% and 71.5% in the second and third doses respectively, adhered to the vaccination scheme.



**ORAL PRESENTATIONS
PATHOGENESIS**

Moderators: JANE MEGID and RODNEY WILLOUGHBY

OVERVIEW OF THE TISSUE IMMUNE RESPONSE IN HUMAN RABIES CASES TRANSMITTED BY DOG

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Presenter: Elaine Raniero Fernandes

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Host cellular immune response in infection by rabies virus (RABV) is determinant to direct the course and clinical manifestations of the disease, having a crucial role in the eradication of virus, contributing to the induction of humoral response, in order to produce neutralizing antibody, which must be regulated to ensure the survival of the host. This study aimed to provide an overview of the tissue immune response in human rabies cases transmitted by dog in the meningeal, perivascular and parenchymal microenvironments of the CNS. Human rabies cases (N=10) transmitted by dog bites were selected to evaluate the expression of cytokines and cell phenotype in comparison with control group (N = without infection by immunohistochemistry technique. We observed predominance of astrocytes, followed of TCD8 + lymphocytes in rabies group. Significant statistically differences also were observed in the number of B lymphocytes, iNOS, apoptotic cells and cells expressing cytokines TGF-beta, IL-23, IL-12, IL-17 and IL-6 suggesting the establishment of a Th17 profile of immune response in rabies group. The low expression of inflammatory cytokines (e.g., TNF-alpha, IL-1 beta) and Th1 cytokines profile (e.g., IFN-gamma) also was observed. Our data indicate that immune response profile of human rabies infection transmitted by dog have a low production of Th1 and proinflammatory cytokines, on the other hand, a greater expression of Th17 cytokine profile in attempt of viral clearance. The high number of astrocytes could be contributing to the production of cytokines such as IL-12 and IL-23, emphasizing the role of resident cells in rabies. In addition, our result suggests that the IL-23 could be maintaining of Th17 profile, as well as to interfere with in the JAK-STAT signaling and in the production of IFN-gamma.

DISTRIBUTION OF PATHOLOGIC CHANGES IN BRAIN OF HORSES WITH RABIES FROM ARGENTINA

Micheloud JF., Jauregui G., Medina DM., Delgado F.

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Presenter: Fernando Delgado

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The infection by Rabies Virus develops a nonpurulent inflammation of the Central Nervous System in many animal species, and Negri bodies can be detected within the cytoplasm of neurons. In horses, previous studies suggested that pathologic affection of brain may be lower than spinal cord, and changes may be not present. A recent report described that changes of rabies of cattle naturally infected from Argentina were not exactly than those described in other countries, and it could be related with a different type of virus, the dose inoculated or the course of the disease. The aim of this study was to evaluate the pathologic changes developed in the CNS of naturally infected horses from Argentina. Formalin fixed-paraffin embedded samples of brain (cortex, basal nuclei, thalamus, midbrain, brainstem, cerebellum and cervical spinal cord) of 3 horses positive for rabies were analyzed by histopathology. Two animals showed nonpurulent meningoencephalitis, multifocal gliosis and small hemorrhages in one or more areas of brain. The other animal, from which spinal cord was not available for inspection, did not show pathologic changes. Spinal cord did not show changes in the animals with encephalitis. No animal had Negri corps. The changes found are similar than those described by other reports, but distribution was not the same, since affection of spinal cord did not occur. The low number of animals analyzed makes impossible to get some conclusions. However, the different distribution of changes than the reported in other countries may suggest that pathogenesis could be different in Argentina. Further studies should be performed to determine the origin of the differences found.

**ORAL PRESENTATIONS
HUMAN RABIES AND PROPHYLAXIS**

Moderators: ALAN JACKSON and CRISTIÁN BISCAYART

Willoughby, Rodney E.

Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Presenter: Rodney E. Willoughby

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Background: The burden of human rabies continues despite promising improvements in vector control and human and animal prophylaxis.

Methods: Literature review, treatment registry

Results: There are 93 known uses of the Milwaukee Protocol (MP), with 18 survivors of human rabies. While survivors are increasingly reported using conventional critical care, most known survivors are associated with the MP (18/32). Survival statistics for human rabies vary by rabies source and continent, and prior prophylaxis. Peru and Brazil have the most rabies survivors. The highest survival rates follow vampire bat exposures (24%); dog rabies is intermediate (21% Americas; 12% Asia; 0% Africa). The worst survival (0%) follows North American bat exposures. Survival is higher with failed prior prophylaxis for dog and bat rabies. However, among all survivors (MP, other), good outcomes are solely associated with lack of prior PEP (OR 5.3, CI 0.77-42). The mechanism for this non-competing set of risks is unknown: neutralizing antibody titers in serum and CSF of survivors do not correlate with prior PEP or functional outcomes. Logistics of diagnostics, drug supply and human subject protection remain major barriers to rabies research and treatment.

Conclusion: Survival from human rabies is regular. Survival and function do not divide cleanly between bat and canine rabies. Prior PEP results in more survivors but worse functional outcomes; immune correlates are lacking but needed to explain the effect of prior PEP. "Rabies kits" developed under regional public health agreements may offer a way forward.

DEVELOPMENT OF SYN023 ANTI-RABIES MONOCLONAL ANTIBODIES

Eric I. Tsao and J. Bruce McClain
Synermore Biologics

Presenter: Eric I. Tsao

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SYN023 is a mixture of two anti-rabies humanized monoclonal IgG1k 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. Phase 1 and Phase 2 human clinical trials were conducted in the U.S. to evaluate the pharmacokinetics, pharmacodynamics, and safety of the product. A Phase 1/2 clinical trial is ongoing in China. The clinical trials data and global regulatory considerations will be presented.

BAT-TRANSMITTED HUMAN RABIES: INFORMATION SOURCES AND KNOWLEDGE IN CURUÇÁ, BRAZILIAN AMAZON REGION

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Presenter: Marcela Arias Caicedo

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The municipality of Curuçá, in the Brazilian Amazon, has natural reserves, where the extractivism is part of the population routine. Humans aggressions by bats are common during the extractivism activities. The objective of this study was to verify what the population of Curuçá knows about the transmission, prevention and risks of rabies and, to observe the means used by the population to get informed. Three hundred and seventy-one individuals answered a semi-structured questionnaire (sample size calculated in EpiInfo™ software 7) to evaluate their knowledge and attitude on this topic. A proportionate stratified random sampling was used, considering the census tracts as stratum. The interviews were carried out from October 2017 to March 2018. As preliminary results of the 371 questionnaires already analyzed, bats were the second most involved animal in the aggressions reported by the subjects (22.7%). In the first place were the dogs (74.2%). More than half of the individuals (52.6%) reported having experienced some type of animal aggression, but the majority (54.9%) did not seek for health service, mainly because they did not attribute seriousness to the aggressions (32.4%). Regarding knowledge about rabies, 72.4% said they know what the disease is. As for the animals involved in the transmission of the disease, only 25.4% indicated the bat. Regarding the symptoms, 46.1% said they did not know any symptoms. As for obtaining information about the disease, 18.8% said they had never heard about rabies. Of those who had any information, the majority, 34.1%, reported getting the information from informal sources such as family or other community members. 68.5% did not hear about the human rabies outbreaks that occurred in Brazil in 2004 and 2005. These results strongly indicate that informative actions are necessary to improve the population's knowledge about the hazard of bat aggressions and the rabies transmission.

HUMAN RABIES BY SECONDARY TRANSMISSION IN BRAZIL

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Presenter: Silene Rocha

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In recent decades, Brazil has reduced over 90% the human cases of rabies (RABV) caused by dogs and cats, which characterizes the urban cycle of the disease, however there has been an increase in the detection of rabies in the wild cycle. Other different wild rabies variants were isolated in the country, mainly the hematophagous bats *Desmodus rotundus* (AgVMH) in different mammals, among them non-hematophagous bats, monkeys, herbivores and in dogs and cats in urban areas. This circumstance may lead to the transmission of RABV to humans, defined as secondary transmission. This study aims to describe cases of rabies in humans by secondary transmission in Brazil from 2001 to 2017. The descriptive study covering the period from 2001 to 2017 evaluated the reports of registered human rabies from the Information System of Complaint and Notification. There were 162 cases of human rabies transmitted by different mammals from the wild and domestic cycle. The AgVMH rabies variant was detected in 57% (92/162) of the human cases. Of these 51% (82/162) had the hematophagous bat as an aggressor and 6% (09/162) recorded AgVMH caused by other mammal. Domestic cats were aggressors in 3% (04/162) of the human cases with the presence of the bat variant hematophagous, 2% (3/162) by unidentified mammals and 1% (2/162) by herbivores. The records of secondary human rabies evidenced the interaction dynamics of RABV between wild and domestic mammals, such as dogs and cats, related to the adaptation of the virus to other species (bat - cat - man). They demonstrated ruptures in the ecosystem equilibrium, with the aggravating reintroduction of the disease in the urban cycle. Therefore, greater attention is needed in surveillance and prevention actions in the wild cycle of the disease, which is growing in the country.

SAFETY AND NEUTRALIZING RABIES ANTIBODY TITERS IN HEALTHY SUBJECTS GIVEN A SINGLE DOSE OF RABIES IMMUNE GLOBULIN CAPRYLATE / CHROMATOGRAPHY PURIFIED

Kim Hanna; Maria Cristina Cruz; Elsa Mondou; Edward Corsi; Peter Vandeberg - Grifols

Presenter: Elsa Mondou

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Rabies immune globulin (RIG) and vaccination series are necessary for postexposure prophylaxis. A new formulation of RIG (human) purified by caprylate/chromatography (RIG-C) was evaluated and recently published.¹ This formulation is twice as concentrated (300 IU/mL) as other hRIG preparations, thus half the injection volume is needed to achieve the standard dose of 20 IU/kg following rabid animal exposure, and the reduction in volume may allow the physician to infuse a higher proportion of the RIG dose into the bite site. **Objective/Methods:** This open-label, single-arm study in healthy subjects evaluated neutralizing rabies antibody concentrations produced from a single 20 IU/kg intramuscular (IM) dose of RIG-C measured by rapid fluorescent focus inhibition test (50% neutralization endpoint) 1-hour postdose and on days 1, 2, 4, 6, 8, 10, 14, 18, and 21. **Results:** Twelve subjects enrolled. No discontinuations, serious adverse events, or treatment-emergent clinically significant changes in laboratory parameters were observed. All adverse events (AEs) resolved and were mild except 1 moderate AE of oropharyngeal pain. Injection site pain (4 subjects) was most commonly reported. RIG-C produced a rapid increase in neutralizing rabies antibody: mean value 0.113 IU/mL 24 hours after IM injection, peak on day 4 (0.132 IU/mL), persisting through day 21 (0.116 IU/mL). The mean reciprocal titer was 11.5 by day 2; peak was 12.1 on day 4; and mean ≥ 10.6 was maintained through day 21. **Conclusions:** RIG-C was well tolerated and provided neutralizing rabies antibodies, which combined with vaccine series after rabies exposure, should result in effective prophylaxis per World Health Organization/Centers for Disease Control and Prevention guidelines. RIG-C should provide an immunologic bridge until active vaccine-induced neutralizing antibodies (≥ 0.5 IU/mL) are produced approximately 7-10 days after the first vaccine dose.

Recent publication: Hanna K, Cruz MC, Mondou E, Corsi E, Vandeberg P. Safety and neutralizing rabies antibody in healthy subjects given a single dose of rabies immune globulin caprylate/chromatography purified.

Clinical Pharmacology: *Advances and Applications*. 2018; 10: 79–88. [Submitted to RITA 2018 conference with permission from Dove Medical Press Limited, reference GRI181424].

Trial registration: ClinicalTrials.gov identifier is NCT02139657.

Keywords: rabies, rabies immune globulin, RIG-C, prophylaxis, rabies neutralizing antibody titers, GTI1301".

ASPECTS RELATED TO HUMAN RABIES ATTACKS ATTACKED BY BATS IN THE STATE OF CEARÁ/BRAZIL, 2007-2018

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Due to man's intervention in the environment, the population of bats in urban and rural areas of Brazil has increased in recent years, with increased risk of aggression and the transmission of rabies to humans. Objective: To characterize the epidemiological profile of human rabies infestations resulting from bat attacks in the state of Ceará, from 2007 to 2018. Methods: The study is descriptive and cross-sectional, based on the records of the human rabies control of the Notification of Injury Information System. The absolute and relative frequencies of socio-demographic variables (sex, age group, area of residence) and specific bat cases were analyzed (kind of aggressor, type of accident, location of injury, type of treatment). Results: In the analyzed period, there were 321,093 human rabies cases, of which 1,737 (0.6%) were bat-related attacks recorded in 169 municipalities (92%) of Ceará. The reports were more frequent in males (945, 54%) and in the age group of 20 to 29 years (319, 18%). The highest number was recorded in residents of the urban area (1,188, 69%), with 310 cases (18%) in the capital Fortaleza. The bite was the most present type of aggression (1,434; 83%), mainly in the hands / feet (871; 50%). However, serum prophylactic conduct along with vaccine was prescribed in 1,160 (67%) cases. Discontinuation of anti-rabies treatment was observed in 11 (0.7%) patients; of these, eight (72%) abandoned treatment.

Conclusion: In view of the high number of bat attacks and failure to indicate prophylactic behavior, it is necessary that health professionals follow the Technical Norms of the Ministry of Health and act in an integrated way to strengthen the surveillance of rabies in the state of Ceará.

Keywords: Aggression, bats, post-exposure prophylaxis, rabies.

**ORAL PRESENTATIONS
VACCINES AND ANTIVIRALS**

Moderators: RICHARD B. CHIPMAN and OSCAR PEREZ

SMALL ANTIVIRAL COMPOUNDS FOR RABIES: A CHALLENGE BY A SINGLE-APPROACH METHOD IN TREATMENT

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Presenter: Wu Xianfu

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After clinical symptoms are manifested, rabies patients are considered untreatable beyond palliative care, despite gains in knowledge made over the years following inception of the experimental Milwaukee Protocol (MP). Small antiviral compounds such as ketamine, amantadine, and ribavirin (no longer recommended) have been part of the comprehensive countermeasure regimen in the MP. Since 2011, CDC has partnered with Prosetta to find small compounds, with MWs less than 500 Da that are highly potent against rabies virus growth in vitro. From about 1000 candidates generated by 45 iterations of chemical modifications at Prosetta, we selected 4 compounds that arrested the release of a recombinant rabies virus (ERA-NLS/GFP) in cell culture, i.e., no virus detectable in cell supernatants, and no detectable expression of green fluorescent protein (GFP). While within the cells, we observed the "bullet-shaped" virions by electron microscopy. When the antiviral compounds were removed from treated cells which were further cultivated an additional 3 to 5 days, GFP became visible and the virions were detectable in cell culture supernatants. We hypothesize these antivirals work by inhibiting virus release through a proposed "aberrant assembly machine" (2013 PNAS), but don't present a mechanism on how the "infected virus/genome" within the cells will be cleared (A presentation by Todd Smith will demonstrate the select compounds did not work in rabies treatment in animal models). Many commercial antivirals, including those for HIV, herpesviruses, hepatitis, and influenza, work by reducing virus load, but don't support "virus clearance" after infection. Our single-approach method in finding antiviral compounds for rabies treatment will be a challenge for in vivo or clinical studies, yet incorporating them into comprehensive countermeasures like the MP could be an alternative".

THE HUNT FOR RABIES ANTIVIRAL DRUGS

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Presenter: Todd Smith

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Rabies in humans is completely preventable with proper, timely post-exposure prophylaxis. However, tens of thousands of people die each year worldwide due to multifaceted barriers to rabies prophylaxis. As healthcare standards increase around the world, the demand for a rabies antiviral therapeutic agent is of the highest importance. We previously reported on TAM004 (Tamir Biotechnology, Inc.), a type III RNase that was originally developed for oncology, which has potent, wide spectrum antiviral activity that includes rabies virus. We also previously reported on small molecular weight compounds (Prosetta Antiviral Inc.) that were selected for rabies antiviral activity using a cell-free protein synthesis system. In both cases, the candidate drugs strongly inhibited rabies virus in cell culture. Here we report on in vivo trials of these drugs in the canine-rabies-virus-challenge, Syrian-hamster model. A single drug dose was selected based on previous in vivo antiviral and toxicology studies involving each drug. For TAM004, 0.1 mg/kg of the drug was administered beginning 24 hours before intramuscular rabies virus infection and continuing once per day for up to 10 days (the duration of the virus incubation period). The drug was administered by intraperitoneal, intramuscular, and intravenous routes. For the small molecular weight compounds, 0.2 mg/kg of the drugs was administered intraperitoneally beginning either 2 or 7 days after intramuscular rabies virus infection because more data on brain penetration was available. Although the animals tolerated the different drug treatments well, none of the drugs tested had a significant effect on rabies survival compared to controls. While these initial results are not promising, these studies provide valuable information to direct future drug design and selection such as modifications to improve permeability through the blood brain barrier, improvement in bioavailability, and using multiple drugs to attain a synergistic effect.

ARE NOVEL VACCINES NEEDED FOR NOVEL VIRUSES?

Ashley C. Banyard, Guanghui Wu, Rebecca Shipley, David Selden, Lorraine M. McElhinney, Anthony R. Fooks

Presenter: Ashley C. Banyard

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Rabies virus (RABV) causes over 59,000 human deaths globally every year and belongs to the lyssavirus genus, a group of viruses that cause 100% fatality following the establishment of symptomatic infection. Alongside RABV, the genus consists of a number of genetically related viruses that are proposed to be able to cause fatal encephalitis clinically indistinguishable from rabies. Whilst the human burden of these non-rabies lyssaviruses remains unclear, they have been responsible for 13 human fatalities. Lyssaviruses continue to be isolated from different species globally, although most commonly, novel isolates are described in bats. Since the turn of the century, evidence for 10 distinct lyssavirus species has been reported, taking the total number of lyssaviruses within the genus to 16. Two further viruses await official classification. The discovery of novel lyssaviruses raises concerns regarding vaccine protection. It has been reported that existing human and animal rabies vaccines, all based on RABV, are not able to elicit a protective antibody response that neutralises all lyssaviruses. Tools to combat rabies virus through either pre-immunisation of individuals (vaccines) or the treatment of exposure events (vaccines and rabies immunoglobulin) have been available for decades. Despite these tools, preventable rabies deaths still occur, predominantly through cost, a lack of availability and the need for multiple clinic visits in areas where the virus is endemic. Therefore novel vaccine formulations are required. The lyssavirus glycoprotein is the sole target for virus neutralising antibodies induced by vaccination and targeted by rabies immunoglobulin yet divergent lyssaviruses are not neutralised by these preparations. Here we describe the current situation regarding the continued discovery of novel lyssaviruses and the challenges to the development of tools to counteract potential infection with antigenically divergent members of this important group of viruses.

THE EARLY USE OF PASTEUR'S RABIES VACCINE IN THE UNITED STATES

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Presenter: Alan C. Jackson

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Louis Pasteur's rabies vaccine was first administered for rabies post-exposure prophylaxis to Joseph Meister in Paris in July 1885. A year later the vaccine became available within the United States. In June 1886 Harold Newell was bitten by a rabid dog in New Jersey. Eleven days later he received the first dose of Pasteur rabies vaccine in New York City, which was supervised by surgeon Dr. Valentine Mott at the first Pasteur Institute in America. Subsequently, Harold received three further doses of the vaccine, but he did not complete a course of 10 planned inoculations due to a series of accidents. Harold Newell apparently did not develop rabies and this rabies vaccination received much attention in newspapers across the United States. Dr. Mott had traveled to Paris in March 1886 and visited Pasteur's laboratory during the month of April and learned the techniques for rabies immunization. He returned with a rabbit inoculated with rabies virus from which he was able to propagate the virus and prepare infected rabbit spinal cords for preparation of the vaccine after desiccation. Altogether about a dozen patients were vaccinated at the institute, but it was forced to close in 1887 due to a lack of public and financial support. Another Pasteur Institute opened in New York City in 1890 under the direction of Dr. Paul Gibier, who had completed his doctoral thesis on rabies at the University of Paris, and this institute remained open until 1918. The New York City Department of Health took over the role of distributing rabies vaccine to hospitals and physicians. Highlights of Pasteur's landmark studies on rabies will be presented, including research that pioneered the field of virology and the use of immunization to prevent infectious diseases.

RECOMBINANT MODIFIED VACCINIA VIRUS ANKARA EXPRESSING RABIES GLYCOPROTEIN INDUCES PROTECTION AGAINST CHALLENGE IN MICE

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Presenter: Débora Garanzini

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Inactivated vaccines against rabies are effective but present several disadvantages such as uncertain antigen composition, manipulation of the pathogen during the vaccine manufacturing, need of cold chain during storage and transportation and inability to differentiate vaccinated from infected animals. To overcome these inconveniences, the actual tendency is the rational design of recombinant immunogens (viral vectored or subunit vaccines) that are safe and efficacious against pathogens. The aim of this work is the development and evaluation of an anti-rabies vaccine candidate based on recombinant Modified vaccinia Ankara Virus (MVA).

In order to obtain recombinant MVA viruses we constructed a transfer vector (VT-GUS/RG) carrying the coding sequence of rabies glycoprotein (RG) flanked by genomic regions of the MVA086R gene to allow in vivo recombination. Recombinant MVA viruses expressing RG protein were obtained by transfecting VT-GUS/RG into primary cultures of chicken embryo fibroblast (CEFs) previously infected with MVA. Recombinant MVA-RG was isolated based on its capacity to produce blue-plaques in the presence of b-glucuronidase (GUS) substrate. The presence and expression of RG coding sequence was confirmed by PCR and Western blot, respectively. Then, BalB/C mice were inoculated twice with recombinant or non-recombinant MVA vectors and specific immune response was evaluated by ELISA. The immunization with MVA-RG induces anti-rabies antibodies that increased after the boost and remained stable for at least five months. The IgG2a/IgG1 ratio was 2.72 indicating a Th1-immune response. Next, the protection induced by MVA-RG was evaluated in mice intra-cerebrally infected with rabies virus, at 14 days or 5 months after the second immunization. The percentage of protection was 60 and 80%, respectively.

In this work we obtained a recombinant MVA vector that express RG protein. Besides we demonstrated that MVA-RG induces long lasting immune responses that protect mice against rabies virus challenge.

NEW CHROMATOGRAPHICALLY PURIFIED VERO CELL RABIES VACCINE DEVELOPED IN ARGENTINA

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The present work summarizes the development of a new chromatographically purified Vero cell rabies vaccine. In previous reports we showed the different steps of the development process of this vaccine, from the viral adaptation to the cell substrate, through the scaling up in the upstream and downstream steps. The purified, inactivated and lyophilized virus conserves its antigenicity in the NIH potency assays, fully complying with the European Pharmacopoeia requirements in terms of purity and stability for at least 18 months at 2-8°C. These encouraging results will most probably support a 24-month shelf life for the finish product.

All the results and analysis have been submitted to ANMAT for approval. To our knowledge this is the first report of a human rabies vaccine produced under international standards, entirely developed by a private company in Latin America, contributing to the goal of promoting local vaccine accessibility in the region in order to be applied both for pre- and post-exposure prophylaxis, preventing the disease in humans.

ABHAYRAB: ONE OF THE PIONEERS IN THE GLOBAL CONTROL OF RABIES

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Abhayrab, manufactured by Human Biologicals Institute (A division of Indian Immunologicals Limited), India, is a Purified Vero cell cultured Rabies vaccine (PVRV) - world's second and India's first PVRV. Since its launch in the year 2000, it has been playing a prominent role in the prevention and control of Human rabies in India and in different countries across the globe. Abhayrab is sold in 34 countries besides India, catering to the needs of Asian, Middle East, Africa and CIS countries. More than 80 million doses have been sold since its launch.

The safety and immunogenicity of Abhayrab has been evaluated by several clinical trials in India and abroad. These trials include one sponsored by Human Biologicals Institute as well as those done by independent experts/institutions. The trials include vaccination by intradermal route and intramuscular route and also for pre-exposure prophylaxis and post-exposure prophylaxis. Some special studies including those on pregnant women and in fox bite cases have also been carried out. All these trials proved the safety and immunogenicity of Abhayrab vaccine. Summary results of these trials demonstrate that when administered by intramuscular route, the range of GMT for the anti-rabies antibodies was 3.16 to 17.29 IU/ml on day 14 and 3.16 to 14.16 IU/ml on day 30 post-vaccination. Similarly, when administered by intradermal route, it was observed that, the range of GMT was 3.3 to 7.25 IU/ml on day 14 and 2.68 to 11.04 IU/ml on day 28 post- vaccination.

With a history of more than 18 years of usage, having successfully served markets across the globe by having offered a safe and efficacious Purified Vero Cell Cultured rabies vaccine at competitive prices, Abhayrab wishes to work towards the WHO target of ending human deaths from dog-mediated rabies by the year 2030.

**ORAL PRESENTATIONS
RABIES CONTROL**

Moderators: LAURA ROBINSON and GABRIELA CALAMANTE

ORAL VACCINATION OF DOGS AGAINST RABIES: BAIT ACCEPTANCE STUDIES IN GOA STATE, INDIA

Adriaan Vos, Andy Gibson, Gowri Yale, Julie Corfmat, Parveen Ohal, Thomas Müller, Rupesh Rhane, Steffen Ortmann.

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Oral rabies vaccination (ORV) has been successfully used to eliminate rabies from certain wildlife reservoir species, like red foxes, coyotes and raccoon dogs, from large areas. Consequently, this approach has been suggested as a supplementary tool for vaccination of free-roaming dogs inaccessible for traditional parenteral vaccination. Recently, the concept of ORV of dogs has gained renewed interest and organizations like WHO and OIE have published detailed minimum requirements for candidate oral rabies vaccines and – baits. Baits should not only be well accepted by the target population but also be able to release the vaccine efficiently into the oral cavity. It has been postulated that most likely a universal acceptable bait may not be feasible since bait acceptability depends on the food preferences of the local dog population. However, recent field studies in different countries have shown that a novel egg-based bait was not only very attractive but also effective in delivering the vaccine in the oral cavity of free-roaming dogs. Hence, this bait was also tested in Goa State, India, together with an alternative bait matrix using local available pet food (gravy). A total of 210 and 195 free-roaming dogs were offered the egg - and gravy-bait containing a soft blister filled with blue-dyed water, respectively. No difference ($p=0.99$) was found in the percentage of dogs interested in the baits when offered; egg-bait – 81.4% and gravy-bait - 82.1%. However, significantly more dogs consumed the egg-bait than the gravy-bait; 79.7% versus 70.5% ($p=0.04$). Evaluating bait handling in terms of the release of the blue-dyed water in the oral cavity, 85.8% of the dogs consuming an egg-bait were considered 'vaccinated' compared to only 67.8% of the dogs that consumed a gravy-bait ($p=0.001$). The egg-based bait seems to be universally attractive for dogs living in different settings.

SUB-REGIONAL APPROACH TO DOG-MEDIATED HUMAN RABIES ELIMINATION IN THE SOUTHERN AFRICAN REGION

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Dog-mediated human rabies elimination from the Southern African Development Community (SADC) region, comprising sixteen countries mostly with endemic rabies, will require a regional One Health approach. Southern Africa has the capacity to lead the way in dog-mediated human rabies elimination in Africa with several countries making significant progress towards the “Zero by 30” goal. These countries include Namibia, Tanzania, Mozambique and South Africa specifically the province KwaZulu-Natal. Evaluating the countries Veterinary Services dog-mediated rabies elimination capacities and how best to support sustainable elimination plans could be examined through the OIE tool for the Evaluation of Performance of Veterinary Services (PVS). Working with countries to identify gaps in National Rabies Elimination Plan delivery including rabies diagnostic capacity and data sharing with the Public Health sector will benefit all countries in the region given the transboundary nature of rabies. The OIE lab-twinning project potentially will build diagnostic and surveillance capacity within the region and cross boundary collaborations can greatly benefit countries.

Programmed management with good National Coordinators and engagement of stakeholders appear to be essential elements in official programs in the region. The International collection of rabies data is currently by the OIE World Animal Health Information System (WAHIS) and the District Health Information Software (DHIS2) Human Health sector data capture system. The WHO integrated data platform and WAHIS Plus are new systems on the horizon that will assist in enhancing regional rabies knowledge, planning and response. The PARACON Rabies Epidemiological Bulletin tool of Global Alliance for Rabies Control (GARC) also provides a recent reporting platform across the region. Southern Africa can learn much from the Latin American and Caribbean countries and their Regional Programme for Rabies Elimination but the sub-region will need to accelerate efforts to eliminate dog-mediated human rabies by 2030.

ESTIMATING THE SIZE OF DOG POPULATIONS IN TANZANIA TO INFORM RABIES CONTROL

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However, dog population sizes are generally unknown in most rabies-endemic areas. Several approaches have been used to estimate dog populations but without rigorous evaluation. We compare post-vaccination transects, household surveys and school-based surveys to determine which most precisely estimates dog population sizes. These methods were implemented across 28 districts in southeast Tanzania, in conjunction with mass dog vaccinations, covering a range of settings, livelihoods and religious backgrounds. Transects were the most precise method, revealing highly variable patterns of dog ownership, with human: dog ratios ranging from 12.4:1 to 181.3:1 across districts. Both household and school-based surveys generated imprecise and sometimes inaccurate estimates, due to small sample sizes in relation to the heterogeneity in patterns of dog ownership. Transect data were subsequently used to develop a predictive model for estimating dog populations in districts lacking transect data. We predicted a dog population of 2,316,000 (95% CI 1,573,000-3,122,000) in Tanzania and an average human: dog ratio of 20.7:1. Our modelling approach has the potential to be applied to predicting dog population sizes in other areas where mass dog vaccinations are planned, given census and livelihood data. Furthermore, we recommend post-vaccination transects as a rapid and effective method to refine dog population estimates across large geographic areas and to guide dog vaccination programs in settings with mostly free-roaming dog populations.

THERMOTOLERANCE OF AN INACTIVATED RABIES VACCINE

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Rabies continues to threaten human lives across the world and, despite the availability of effective vaccines, is responsible for an estimated 60,000 deaths annually, nearly all resulting from bites from infected domestic dogs. When mass domestic dog rabies vaccination campaigns achieve 70% coverage the incidence of rabies can be controlled. However, campaigns are costly and time-consuming, particularly transporting vaccines long distances whilst maintaining “cold-chain” conditions (4°C). Coverage is thus limited by proximity to areas with electricity and refrigeration, limiting the scope of campaigns.

Thermotolerant vaccines, stored without loss of potency at ambient temperatures for extended periods, can alleviate these constraints, providing opportunities for increasing coverage in otherwise expensive and hard-to-reach communities. This study used a controlled and randomized non-inferiority trial to investigate whether the Nobivac® Rabies vaccine, commonly used in domestic dog vaccination campaigns, is thermotolerant. Specifically, whether the immunological response in Tanzanian domestic dogs at four weeks following vaccination with doses stored under non-cold-chain conditions (vaccine stored at temperatures between 25-35°C) was not inferior to that elicited following vaccination with doses stored under cold-chain conditions (4°C).

The outcome was that the serological response of dogs vaccinated with Nobivac® Rabies vaccine stored for up to six months at 25°C and up to three months at 30°C was not inferior to that of dogs vaccinated with vaccine stored within the cold-chain.

These findings suggest that this vaccine can be used to access remote hard-to-reach areas that lack the infrastructure to maintain cold chain storage. Additionally, vaccines can potentially be stored in these remote communities allowing dogs, particularly new puppies and dogs introduced in the area, to be vaccinated “on-demand” throughout the year. This would likely lead to broader and more consistent coverage. These findings are important given the global ‘Zero by 30’ commitment and the development of national rabies elimination strategies.

SURGICAL STERILIZATION OF DOGS AND CATS AS A MASSIVE PUBLIC HEALTH STRATEGY IN MEXICO

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In the last decade of the last century, the Ministry of Health began with the National Weeks of anti-rabies vaccination in dogs and cats, noting a considerable increase in the number of dogs to be vaccinated, which favored a proactive dialogue with civil society groups on the strategies for their population control, deciding to implement surgical sterilization in the most complete free way (Ooforosalingohysterectomy in females and bilateral orchiectomy in males). Consider the shared responsibility between the Ministry of Health, State Health Services, State and Municipal Authorities, Schools of Veterinary Medicine and Organized Groups of Civil Society under a "Shared Responsibility Model".

In 1994, only 10 entities participated, gradually adding up the rest of the country, sterilizing in the period from 1994 to 1999 about 104 thousand animals. From 2000 to 2012, 2.4 million dogs and cats were sterilized for free. Currently in 2013-2018, surgeries amounted to 3.5 million; 38% dogs and 32% cats, corresponding 69% to females and 31% males, of these 41% under 1 year and 59% higher.

Mexico is the first country in America that officially allocates resources for these activities with a sanitary focus, providing inputs from the central level for 1.7 million sterilizations (\$ 60.3 million pesos) accumulated from 2001 to date, with 2,882 people participating directly (559 veterinary surgeons) in itinerant days in villages of the country, in fixed days in canine care centers or during the National Sterilization Month (October). Staff of the Ministry of Health performs 61% of sterilizations, 27% personnel hired by local authorities and 12% by animal protection groups.

This activity alone is not considered a strategy for controlling rabies, however, in the case of Mexico since its implementation has contributed as a cross-cutting strategy to the penetration of others such as rabies vaccination and the responsibility of owners with the sanitary care of dogs and cats, being important so that today canine rabies is in the process of elimination.

RABIES CONTROL AND PROPHYLAXIS IN SRI LANKA

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Rabies is still a public health problem in Sri Lanka like in many other countries Asia and Africa. Although effective and safe vaccines are available for people and animals still rabies is accountable for one of the biggest expenditure for a single disease in country's health budget. Medical Research Institute which was known as Bacteriological Institute started producing sheep brain vaccines as early as 1900 in Colombo, Sri Lanka. Human rabies was made notifiable disease in 1971. Human diploid cell vaccines were imported in 1986 and Ministry of Health took a policy decision to stop production of nerve tissue vaccines in 1995. Intra dermal use of rabies vaccines were started in 1997.

In 2005 First Rabies treatment unit was established in the National Hospital of Sri Lanka, which became the model unit and the training centre for Rabies PEP.

Human rabies vaccines are available even at village level rural hospitals. Equine rabies Immunoglobulin is available at Base hospitals and Human rabies Immunoglobulin is provided only a one hospital in each province. Treatment are given free of charge and maximum travel time is around 30 minutes to a ARV centre and 2 hours to a rabies unit. With all existing free health-facilities and having literacy rate of 92% and life expectancy of 74.95 human rabies deaths are have never reached below 19.

Rabies deaths are mostly in adult men who do not seek treatment following an exposure.

Island wide rabies control program was started in 1975. The public health veterinary services are responsible for rabies control and dog vaccination program. Animal health and production is planning to take over dog vaccination program from this year onwards. From 400,000 in 1990, 1.5 million dogs were vaccinated against rabies in 2015. Sri Lanka practices No Kill policy for free roaming dogs.

IMPACT OF COMMUNITY-DELIVERED SMS ALERTS ON DOG-OWNER PARTICIPATION DURING A MASS RABIES VACCINATION CAMPAIGN, HAITI 2017

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Since at least 2011, Haiti has vaccinated between 100,000 and 300,000 dogs annually against rabies, however national authorities have not been able to reach and maintain the 70% coverage required to eliminate the canine rabies virus variant. Haiti conducts massive dog vaccination campaigns on an annual basis and utilizes both central point and door-to-door methods. These methods require that dog owners are aware of the dates and locations of the campaign. To improve this awareness among dog owners, 600,000 text messages were sent to phones in two Haitian communes (Gonaives and Saint-Marc) to remind dog owners to attend the campaign. Messages informing the communes of the campaign dates were delivered on the second day and at the mid-point of the two-week campaign. A post-campaign household survey was conducted to assess dog owner's perception of the text messages, and the impact on their participation in the vaccination campaign. Overall, 147 of 160 (91.9%) text-receiving dog owners indicated the text was helpful and 162 of 187 (86.6%) responding dog owners said they would like to receive text reminders during future rabies vaccination campaigns. In areas hosting one-day central point campaigns, dog owners who received the text were 2.0 (95% CI 1.1, 3.6) times more likely to have participated in the campaign (73.1% attendance among those who received the text vs 36.4% among those who did not). In areas incorporating door-to-door vaccination over multiple days there was no significant difference in participation between dog owners who did and did not receive a text. Text message reminders were well-received and significantly improved attendance at central point campaigns, indicating that short message service (SMS) alerts may be a successful strategy in low resource areas with large free roaming dog populations.

CONTROL DE LA RABIA URBANA EN LA FRONTERA JUJUY (ARGENTINA) - MODESTO OMISTE (BOLIVIA)

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La provincia de Jujuy limita al Norte, a través de 130 km de frontera seca, con la provincia de Modesto Omiste de Bolivia, caracterizada por una altura mayor a 3000 msnm, caminos sinuosos y una población de 0.3 habitantes/Km².

Debido a que Bolivia notifica permanentemente casos de rabia en animales, desde el año 2009, en Jujuy, se realizan campañas de vacunación de caninos y felinos, principalmente en la frontera.

Se realizan 2 tipos de estrategias:

En la ciudad de La Quiaca, con una población de 18750 habitantes, se realiza una campaña intensiva anual de 2 días de duración, generalmente durante sábado y domingo, con la participación de personal del Ministerio de Salud (25 personas - Departamento de Vectores y Zoonosis) y del Municipio de La Quiaca (50 personas).

Se divide el plano de la ciudad en 4 sectores y se numeran las manzanas. Se establecen aproximadamente 30 a 35 grupos de vacunación, formados por 2 personas, a los que se les entrega el material necesario y el plano de las manzanas que debe cubrir. Se trabaja hasta completar el área urbanizada de la ciudad. Los domicilios que no pudieron ser cubiertos se anotan en una planilla para que el municipio complete la vacunación en las siguientes semanas.

En el área de frontera, la población es de alrededor de 2.500 habitantes distribuidos en pequeños poblados y parajes, dedicados a la cría extensiva de ovinos y camélidos cuyos perros son pastores del ganado.

La campaña del área rural requiere alrededor de 5 días, se realiza con 15 personas de los programas de Zoonosis y Vectores y se vacunan entre 650 y 700 animales en total.

Debido a la aplicación de las mencionadas estrategias, desde el año 2009, no se registran casos de rabia animal en Jujuy en la frontera con Bolivia.

**ORAL PRESENTATIONS
DOMESTIC ANIMAL RABIES**

Moderators: VERONICA GUTIERREZ, GABRIEL CICUTTIN
and MARCO VIGILATO

Stephanie A. Shwiff, Vienna R. Brown, Thu Trang Dao, Julie Elser, Hoang Xuan Trung, Nguyen Ngoc Tien, Nguyen Thi Huong, Nguyen Thi Thanh Huong, Arthorn Riewpaiboon, Steven Shwiff, David Payne.

Presenter: Stephanie A. Shwiff

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The global economic impact of canine rabies has been estimated by several studies and results highlight that Asia disproportionately bears the burden of this zoonosis as a result of high levels of human deaths, high rates of post-exposure prophylaxis (PEP), and low investment in preventative dog vaccination efforts. These factors that cause rabies to burden much of Asia, are also present in Viet Nam. This study aimed to estimate the economic burden of canine rabies in a societal perspective including direct and indirect cost of rabies in dogs, livestock, and humans. Using data collected from personal interviews, published literature, published and unpublished reports, and primary data collection, we estimated the economic impact of canine rabies in Viet Nam over a ten year period (2005-2014). We incorporated the direct and indirect costs for PEP, dog vaccination efforts, livestock losses, and disability adjusted life years (DALYs) into the analysis. General findings from this analysis indicate that over the 10 year study period, the total economic impact of canine rabies to Viet Nam was over \$719 million USD. The largest portion of impacts (92%) were made up of PEP related costs over this time period. During the study period, canine rabies impacts created between 36,560 and 45,700 DALYs (measured in years of life lost (YLL)). A total of 914 human deaths were reported over the study period, and deaths/100,000 people were 0.11, which is lower than the reported level for Asian countries. The cost per dog vaccinated was \$1.75 USD. The results of this study indicate that canine rabies impacts in Viet Nam are consistent with the burden reported in Asia, with large expenditures on PEP and very small investments in dog vaccination.

EVALUATION OF FEATURE-EXTRACTED DATA FOR PRIORITIZATION OF RABIES VACCINATION ACTIVITIES IN HAITI, 2017-2018

Benjamin Monroe, Andy Gibson, Fleurinord Ludder, Pierre Dilius, Kelly Crowdis, Frederic Lohr, Julie Cleaton, Melissa Etheart, Marco Vigilato, Max Millien, Ryan Wallace.

Presenter: Benjamin Monroe

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Annual vaccination campaigns in Haiti consistently fail to reach the recommended 70% coverage needed for elimination of canine rabies, leading to the highest burden of human rabies in the Latin American and Caribbean region. Vaccination teams in Haiti are tasked with working in both high-density informal urban settlements and isolated hamlets in the mountainous countryside. Campaign managers must select areas to prioritize for vaccination, as financial and logistical resources are often severely limited. A data-driven method for prioritizing where vaccinations occur would be beneficial for making the process more efficient and objective. This study evaluates one such method, using retrospective data collected from vaccination and surveillance data. Automated feature extraction is a tool that uses machine learning to identify human structures based on their appearance in satellite imagery. A model for human settlements was generated using data extracted from satellite imagery, in conjunction with road connectivity data derived from online open source map platforms. The combined information was used to assign a numeric Settlement Type and Road Connectivity (STARC) code to all communities in Haiti. The STARC code provides campaign managers with a representation of the potential rabies burden and transmissibility for every community. Vaccination points from the 2017-2018 national campaign, which utilized the Mission Rabies smartphone application to record the exact location of each vaccination, were merged with the STARC layer to create a final dataset for evaluation. Overall, 83% of vaccinations occurred in an area identified in the feature-extracted data as suitable for vaccination, based on Spatial overlap. Several areas identified by the model did not receive any vaccinations during the campaign and portions of large settlements were often overlooked, creating potential pockets of susceptible dogs. The synthesis of feature-extracted, epidemiological, and vaccination point data offers a powerful tool to campaign managers for visualizing vaccination coverage and making planning decisions.

VALIDATION AND EVALUATION OF RABIES VIRUS NEUTRALIZING ANTIBODY MEASUREMENT BY THE FLUORESCENT ANTIBODY VIRUS NEUTRALIZATION TEST UTILIZING AN AUTOMATED READER

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Traveling with animal companions to rabies free destinations continues to increase in popularity. Serological proof of adequate response to rabies vaccination by the Fluorescent Antibody Virus Neutralization (FAVN) or Rapid Fluorescent Foci Inhibition Test (RFFIT) is required. Both are serum neutralization techniques that are labor intensive with subjective microscopic readout. The threshold of 0.5 IU/mL is considered adequate by most destinations to prove an immune response to vaccination. The Kansas State University (KSU) Rabies Laboratory tests over 90,000 samples annually via the FAVN and RFFIT methods. The RFFIT has been validated several times at KSU per FDA/ICH guidelines for human testing, but the FAVN has not been similarly validated in our laboratory because it is used strictly for pet travel at KSU and is an OIE published method.

To fully validate the FAVN to the same standards as the RFFIT, a protocol was created focusing on repeatability, accuracy, linear range, lower limit of quantitation (LLOQ), and robustness. Included in the validation was analysis of an automated reading method, using a BioTek Cytation and Gen5 Secure software. The Cytation instrument measures staining intensity of the FITC labeled virus and the Evan's Blue counterstained BHK cells and performs image processing and analysis to count the virus containing cells. The data undergoes further analysis through thresholds set to determine validity of reading, followed by Spearman-Kärber calculation for ED₅₀ and IU/mL of the sample.

After performance of the validation experiments and analysis of results, we conclude the FAVN is adequately precise, accurate, linear, and robust; the LLOQ of the assay is 0.2 IU/mL and the upper limit of quantitation (ULOQ) of the assay is 15.0 IU/mL. Diluability experiments confirm accuracy of pre-dilutions to obtain results >15.0 IU/mL. In addition, the automated reading produced equivalent reading as compared to manual reading on a fluorescence microscope.

DEVELOPING RABIES DIAGNOSTIC CAPACITY FROM BASELINE IN ETHIOPIA: TAKING STEPS TOWARDS CANINE RABIES ELIMINATION

Greenberg L, Orciari L, Hess A, Ansari A, Reynolds M, Wu X, Ellison J, Satheshkumar PS, Olson V, Pieracci E Lauren Greenberg.

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Presenter: Lauren Greenberg

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As an initial step in the implementation of Global Health Security programs, Ethiopia held a workshop to establish 5 priority zoonoses for which to concentrate capacity development activities, prioritizing rabies as their number one country priority. Preventing zoonotic diseases such as rabies requires the implementation of sustainable programs that must be supported by the country's own government over the long-term. Laboratory capacity building, enhancement of the surveillance systems, improved communication between government sectors, community education, mass vaccination campaigns and cell-culture vaccine importation and development are all components of the Global Health Security agenda framework in Ethiopia. The overarching goal of the rabies program is to decrease canine-mediated rabies deaths in humans. Building laboratory capacity for diagnostic testing is integral for Ethiopia's success. Equipping rabies laboratories in the capital as well as two regional labs will allow for incoming sample testing to provide laboratory-based surveillance. In order for Ethiopia to build functioning rabies diagnostic laboratories it was imperative to assess laboratory spaces and provide appropriate supplies and equipment necessary to conduct rabies diagnostic training in order for in country sample testing and reporting to continue. Throughout the process complications involving how samples collection would occur as well as how the sample from the field would be received by the diagnostic laboratories became apparent. Additional complications came from building and facility conditions as well as communication between external partners and cooperating labs regarding equipment ordering and distribution of materials to three lab spaces spread-out throughout Ethiopia. CDC is working with in-country counterparts and other stakeholders to overcome these challenges by readying three working rabies laboratories in country to test suspect rabies samples which will in turn provide information to health care providers for post exposure prophylaxis decisions and a more accurate picture of rabies burden in country from lab based surveillance.



**ORAL PRESENTATIONS
EPIDEMIOLOGY AND SURVEILLANCE**

Moderators: EDUARDO CALDAS and NATALIA CASAS

MONITORING BAT RABIES IN THE UNITED STATES: CONSIDERATIONS FOR IMPROVED SPECIES IDENTIFICATION AND VIRUS CHARACTERIZATION

Emily G. Pieracci, Jennifer A. Brown, David L. Bergman, Amy Gilbert, Ryan M. Wallace, Jesse D. Blanton, Andres Velasco-Villa, Clint N. Morgan, Scott Lindquist, Richard B. Chipman.

Presenter: Emily Pieracci

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Species identification and virus characterization data for rabid bats in the United States is limited. Given the implications that bat rabies virus (RABV) variants have for human health, understanding the transmission dynamics of RABV variants within and between bat species is crucial. To date, there has been limited systematic evaluation of virus characterization data of bats submitted to the U.S. national animal rabies surveillance system. The authors evaluated different categories of specimens and selected those that merit prioritization for virus characterization. These categories, considered samples of epizootiological importance (SEI), were selected based on characteristics that would improve detection of sentinel events in disease dissemination. National animal rabies surveillance data, years 2010–2015, were used to assess the completeness of species and virus identification and to project the additional burden for laboratory testing if all SEI underwent virus characterization. The SEI were defined as: rabid non-indigenous bats; rabid bats in southern U.S. border states, Florida, Puerto Rico and the U.S. Virgin Islands; and rabid bats not commonly found to be infected with RABV. Additionally, we evaluated the number of bats submitted for rabies testing in which species was identified. During 2010–2015, there were 160,017 bats submitted for testing including 74,928(47%) where bats were identified by species. Among the bats with species information included, 33,882(45%) were defined as SEI. Annually, 1,157(95%CI,1,038–1,277) SEI were rabid and 316(95%CI,175–457) underwent virus characterization. Virus characterization of all SEI would result in an estimated increase of 841(95%CI,727–954) samples tested nationally each year. Increased RABV characterization of SEI and identification of bat species would help inform our understanding of RABV ecology in bat species, some of which have frequent human contact. Improved virus characterization of rabid bats in the United States could inform our understanding of transmission dynamics of RABV variants in bat species.

MONITORING RABIES EPIZOOTICS IN THE UNITED STATES: BALANCING THE NEED FOR A SENSITIVE RABIES SURVEILLANCE SYSTEM WITH REASONABLE EXPECTATIONS FOR LABORATORY MONITORING

Emily G. Pieracci, Richard B. Chipman, Clint N. Morgan, Catherine M. Brown, Jordona D. Kirby, Jesse D. Blanton, Andres Velasco-Villa, Ashlee D. Martin, Kathleen M. Nelson, Amber Singh, Elizabeth LeMasters, Zachary Weiner, Ryan M. Wallace.

Presenter: Emily Pieracci

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A standardized rabies virus (RABV) surveillance system that includes virus characterization information could enhance early detection of: host-shift events, translocation of RABV variants, and discovery of novel variants. To date, there has been no systematic evaluation of either the representativeness or completeness of the virus characterization data in animals submitted to the U.S. national animal rabies surveillance system. Categories were developed to define samples of epizootiological importance (SEI) likely to yield actionable or otherwise critical information relevant to rabies control. Specimens from these categories were considered significant relative to host-shift, translocation, novel variant, or epidemiologically unusual events that might affect future rabies management or post-exposure prophylaxis recommendations. SEI were identified by assessing species, geographies, and epidemiologic risk factors for which RABV characterization should be conducted in order to identify events of interest. The 2010–2015 U.S. national animal rabies surveillance data were used to estimate the additional laboratory testing burden if all SEI underwent viral characterization. Twelve specific SEI categories were devised based on the 4 epizootiological events above. They were evaluated for completeness of virus characterization information. During 2010–2015, there was an annual average of 855 (95% CI, 739–971) rabies positive SEI; an average of 270 (95% CI, 187–353) (31.6%) SEI underwent virus characterization. Virus characterization of all SEI would be expected to increase laboratory testing by approximately 585 (95% CI, 543–625) samples per year. Prioritizing RABV characterization of SEI may improve early detection of critical rabies events. Virus characterization of SEI may help refine wildlife rabies management practices. The recommendation for testing of SEI should be weighed by each public health laboratory against the need to ensure testing does not strain routine rabies diagnostic laboratory capacity.

GENETIC DIVERSITY OF RABIES VIRUS IN HAITI AND DOMINICAN REPUBLIC

Rene Edgar Condori¹, Augustin Pierre Dilius², Rolain Cadet², Griselda Lopez³, Anna Kendy Gomez³, Geovanny Molina³, Claire Hartlodge¹, Andres Velasco-Villa¹, Crystal Gigante¹, Victoria Olson¹, Yu Li¹, Ryan Wallace¹.

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Haiti and Dominican Republic (DR) share one of the most densely populated Caribbean islands. Haiti has the highest incidence of human rabies deaths in the western hemisphere, caused primarily by transmission from domestic dogs. Canine rabies is also enzootic in DR; both countries share a common border through the central mountainous range of the island. Phylogenetic analysis of rabies viruses (RV) can reveal relevant epidemiological information, which can be crucial for designing effective rabies control programs. To understand the genetic diversity of RV across Haiti, a collaborative surveillance effort between CDC and Haiti's Ministry of Agriculture was established and animal rabies samples were collected between 2014 to 2017. Recently, rabies surveillance in DR detected rabies in humans, domestic and wild animals and submitted those samples to CDC for further characterization. These samples provided the opportunity to explore RV variant distribution across the island. Fifty-five RV isolates from Haiti and twenty-two from DR were sequenced and underwent phylogenetic analysis. Four distinct clades associated to canine rabies were identified. Clade A was mainly limited to Haiti's Western coastline, while Clade B was found along the border between Haiti and DR. DR presented, at least two unique RV clades, which are distinctive from those circulating in Haiti. Clade C from DR formed a unique cluster in the phylogenetic tree, which included domestic dog and human cases collected near Santo Domingo (2009-2012). Clade D comprised isolates widespread across DR with no clear geographic boundary; this cluster included an isolate from a rabid dog linked to human case in the east coast, and isolates from dog and mongoose from the south coast. Phylogenetic inferences performed in this study provides valuable information on rabies epidemiology in Haiti and DR, which may be used to support the formation of binational rabies control strategies to eliminate canine rabies.

AN EVALUATION OF BRAZIL'S HEALTH INFORMATION SYSTEM FOR SURVEILLANCE AND PREVENTION OF CANINE RABIES

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Presenter: Julio Benavides

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An effective surveillance system is a key component for the elimination of canine rabies. Brazil has made substantial progress towards the elimination of canine rabies, but outbreaks still occur in a few states. Since 1998, the country uses a health information system (SINAN) to record patients seeking post-exposure prophylaxis (PEP) following an animal bite. The aim of our study was to evaluate: (i) whether these data can be reliably used for rabies surveillance (ii) if patients are receiving appropriate PEP and (iii) the benefits of implementing the latest World Health Organization (WHO) recommendations on PEP. Analysing SINAN records from 2008 to 2017 reveals an average of 506,148 bite-injury patients/year [range: 437,923-545,493] in the country, equivalent to an incidence of 255 bite injuries/100,000 people/year [range: 231-280]. We found that, in most states, records of suspect rabies exposures from SINAN do not correlate with positive dog rabies cases reported to the PAHO SIRVERA surveillance database. States expected to be rabies free reported bites by suspect rabid dogs to SINAN and there was a general increase in the number of bites by dogs evaluated as suspected for rabies with time, including in states not reporting canine rabies to SIRVERA in the last decade. Analyses showed that in 2017, only 45% of patients received appropriate PEP as indicated by the Brazilian Ministry of Health guidance. Implementation of the new WHO recommendations for an abridged 3 dose intradermal PEP regimen including a precautionary single dose for dog bites prior to observation would reduce vaccine volume use by 75%, with potential for cost savings of over USD 23 million per annum. Overall, these results highlight the need to improve the implementation of SINAN, including the training of health workers delivering PEP, so that it can be used as a reliable surveillance tool for canine rabies elimination.

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Human and animal rabies have been nationally notifiable conditions in the United States since 1944. National animal rabies surveillance is a laboratory-based system that consists of more than 130 state public health, agriculture, and university laboratories performing the standard direct fluorescent antibody test. In addition, USDA/APHIS/Wildlife Services conducts active surveillance on animals in enhanced surveillance zones primarily surrounding oral rabies vaccination areas using the direct rapid immunohistochemical test. Active surveillance accounts for approximately 5% of all animals submitted for rabies testing. Data submitted by reporting jurisdictions and agencies were analyzed temporally and geographically to assess trends in domestic and wildlife rabies cases. National rabies management decisions, vaccination recommendations, public education, and numerous 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 2017. 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. Human rabies cases from January 2003 through September 2018 are summarized.

Natalia Casas

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The rabies is a fatal zoonotic viral disease that is transmitted to humans through contact (mainly bites and scratches) with infected animals, both domestic and wild. For its health, social and economic character is considered a neglected disease. Aim: describe the epidemiological scenario of rabies in Argentina in the period 2013-2017. Material and Methods: descriptive and retrospective study. The data sources were bases of the National Health Surveillance System. Results: between the years 2013 and 2017, 191 cases of animal rabies were reported in Argentina. Where 84,4% were insectivorous bats; 11,0% dogs; 3,1% cats and 1,0% others wild animals (fox, coati). In the northwestern and northeastern regions circulate rabies in dogs (virus variant 1 and 2) and in all regions of the country circulate rabies in insectivorous bats (variants 4 and 6). Also in the northwestern, northeastern and central regions circulate the variant 3 (rabies in hematophagous bats) that produces paresis rabies mainly in cattle and horses. The last case of human rabies occurred in 2008. Conclusion: In Argentina the rabies is distributed throughout the national territory. Rabies in all animal species and humans is notifiable in obligatory form. The Ministry of Health supports vaccination campaigns in dogs and cats in areas with circulation of variants 1 and 2; also distribute human and veterinary vaccines. For the control of this disease is essential the interdisciplinary and intersectoral work, requiring "one health" approach and involving actors of all the disciplines and of the different Public and Private Systems.

EVALUATING THE RATE AND DIRECTION OF SKUNK RABIES SPREAD IN THE GREAT PLAINS

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Presenter: Ram Raghavan

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Rabies among striped skunks represent a considerable public health threat in North America due to spillover potential to a wide range of other hosts. One of the key aspects of prevention from rabies and ensuring public health safety is to determine if this disease is spreading, at what rate, and in what direction. Further, knowledge of spatiotemporal patterns and potential ecological drivers will help us assess rate and direction of spread. Our previously published work in this area has given us important inputs for parametrizing a rate and direction of spread modeling. In this study, we utilized case submission data received at the Kansas State Veterinary Diagnostic Laboratory over a period of 2013 through 2018 to determine at what rate skunk rabies is spreading and in what direction(s). We used two contemporary approaches for modeling, viz., diffusion, and occupancy modeling. Five-kilometer buffers were used as appropriate size home ranges for positive skunks, and the same buffers were used as primary cells in the predictive modeling. Rabies positive cases were alone used in the modeling, constructed a Bayesian framework. Both diffusion and occupancy models predicted similar rate of spatiotemporal spread of 1.7, 1.8 km, respectively. No seasonality was detected, and the spread was non-significant specific to any one direction – indicating that the virus is expanding its range in all directions year to year and cell to cell. Landscape features are critical in the dynamics of skunk rabies in the Midwest, and management practices may benefit from considering these factors.

**ORAL PRESENTATIONS
WILDLIFE RABIES**

Moderators: RICHARD CHIPMAN and DANIEL CISTERNA

RABIES RESERVOIR IDENTIFICATION OF TERRESTRIAL CARNIVORES THROUGHOUT ETHIOPIA

Laura Binkley, Jeanette O'Quin, Balbine Jourdan, Wondwossen Gebreyes, Getnet Yimer, Laura Pomeroy

Presenter: Laura Binkley

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Throughout Ethiopia, one of the most rabies-affected countries on the African continent, domestic dogs have been identified as the primary reservoir for RABV transmission. However, little is known about transmission in other terrestrial carnivore populations or the existence of maintenance communities. Knowledge of within and between species contact rates can help determine the ability of a population, or a group of populations, to act as a reservoir. This study utilizes data from stationary camera traps placed at communal foraging sites throughout Ethiopia (1-Addis Ababa, 1-Awash, 2-Awassa, 1-Bale) in order to estimate contact rates. Cameras were set to take photos at 1-minute intervals during a 16-hour period (19:00-11:00) over 3-13 days. Data were transcribed by taking the maximum number of paired interactions within a photo averaged over all recording periods for a given site. Results showed significant geographical variation. Averaged across all sites, intraspecies contact rates were highest in dogs followed by spotted hyenas and domestic cats. Interspecies interactions between spotted hyena and cat were the only pair to reach an average of at least 1 when averaged across all sites. Contact rates were applied to an SEIR model using a 3-day infectious period. Results showed that both the spotted hyena and dog populations were able to maintain independent transmission at three out of five sites. Cats were able to maintain independent transmission at one site. Contact rates were then applied to a mathematical expression for the basic reproductive ratio (R_0) for the case of two species, assuming that transmission can occur via within and between species contacts. Results showed that though these interactions occur, they do not occur at a high enough frequency to establish a maintenance community. Results from this study are the first steps in identifying potential reservoir hosts in addition to dogs for rabies transmission throughout Ethiopia.

TWO MODELING APPROACHES FOR UNDERSTANDING THE SPATIAL DYNAMICS OF WILDLIFE RABIES

Laurie Baker, Jason Matthiopoulos, Thomas Müller, Conrad Freuling, Patrick Wysocki, Ronald Schröder, Elias Krainski, Håvard Rue, and Katie Hampson

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Spatial models capable of capturing local infection dynamics in wildlife populations have great potential to improve the planning of control programmes. However, developing models that can capture and explain spatiotemporal infectious processes at a local level remains a challenge due to a lack of high-resolution spatial and temporal data and limited population and surveillance data.

Using fox rabies in Eastern Germany as a case study, we present two modelling approaches that can be used to understand the spatial dynamics of disease and deal with uncertainty inherent in wildlife epidemiological data. In the first approach, we apply a metapopulation model fit using a Bayesian state-space modeling framework to three decades of monthly data from 5 regions (1982-2006) to estimate regional coupling and rabies transmission. In the second approach we develop a space-time model in R-INLA fit to geo-referenced monthly rabies cases pre-vaccination (1982-1990) to capture the spatiotemporal dynamics of rabies transmission and estimated the spatial dependence between rabies cases.

We demonstrate how the Bayesian state-space modelling and latent Gaussian modeling approach in R-INLA can be used to deal with uncertainty inherent in wildlife epidemiological data. Specifically, we show how metapopulation models fit using Bayesian state-space modeling approach can be used to model aggregated surveillance data and is especially useful for planning vaccination campaigns conducted at regional levels. We illustrate how the space-time model fit in R-INLA is able to capture key aspects of spatiotemporal dynamics of rabies transmission and opens up an exciting opportunity to develop strategic guidance for local disease control. Estimates of the spatial dependence between rabies cases derived from this approach allow us to understand the spatial scale of transmission and inform control strategies by identifying areas of high prevalence where there is a greater risk of infection.

VACCINATION OF SMALL INDIAN MONGOOSES (*Herpestes auropunctatus*) WITH ONRAB VIA DIRECT ORAL INSTILLATION – PRELIMINARY RESULTS

Are R. Berentsen, Christine E. Ellis, Israel L. Leinbach, Shylo R. Johnson, Robert T. Sugihara and Amy T. Gilbert

Presenter: Are Berentsen

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The small Indian mongoose (*Herpestes auropunctatus*) is an invasive pest species and rabies reservoir in Puerto Rico. Currently no oral rabies vaccine for mongooses is licensed for use, although research suggests oral rabies vaccination is possible. The Ontario Rabies Vaccine ONRAB (AdRG1.3-ONRAB) is a human adenovirus rabies glycoprotein recombinant oral vaccine currently licensed for use with striped skunks (*Mephitis mephitis*) in Canada, and under experimental use in the United States since 2011. We evaluated the immunogenicity of ONRAB vaccine in captive small Indian mongooses by direct instillation into the oral cavity (DIOC). We delivered 1.0 mL of neat vaccine and two dilutions prepared in culture media to mongooses (5m, 5f) in three treatment groups: 1) 109.5 TCID₅₀/mL, 2) 108.8 TCID₅₀/mL, and 3) 108.5 TCID₅₀/mL. Six control animals (3m, 3f) received 1.0 mL culture media by DIOC. We collected serum samples from all individuals prior to vaccination and at days 14 and 30 post-vaccination (pv). Serum samples were analyzed for rabies virus neutralizing antibody (RVNA) by the rapid fluorescent focus inhibition (RFFIT) test, and titers > 0.1 IU/mL were considered RVNA positive. By day 14 pv, 26/30 vaccinates had seroconverted: Group 1 (9/10; GMT = 10.1 IU/mL); Group 2 (7/10; GMT = 1.3 IU/mL); Group 3 (10/10; GMT = 2.8 IU/mL). By day 30 pv all vaccinates demonstrated increased RVNA (GMTs = 16.3, 9.5 and 5.4 IU/mL; Groups 1, 2 and 3, respectively). All sham vaccinated animals remained seronegative during the study. The results suggest ONRAB is immunogenic by DIOC route for mongooses, even at a log lower dose than typically used in the vaccine bait. Follow-up studies should consider bait delivery of the vaccine to further evaluate the suitability of the current ONRAB bait product for field distribution to free-ranging mongooses.

COMPARISON OF ECOLOGICAL NICHE OF RABIES IN ALASKA, UNITED STATES AND CANADA

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Wildlife rabies poses significant risks to public and animal health in the United States and Canada. In this study we utilize machine learning to data-mine, model-predict, and examine the ecological niche of rabies distribution and detection in Alaska (U.S.) and Canada. We utilize a previously published dataset of reported terrestrial rabies cases in Alaska, and also expand our analysis using a total of 1,713 geo-referenced cases of rabies (terrestrial and bat) reported in Canada from 2007-2016. In Canada's landscape, rabies can be divided into northern and southern cases. The terrestrial southern cases are comprised of fox- and raccoon-variant rabies in the east, and skunk-variant rabies in central and western Canada, whereas bat-variant cases are found throughout the southern region. Skunk-variant rabies is enzootic, whereas raccoon-variant outbreaks are connected with incursions from the U.S. border regions. Cases in northern Canada and Alaska are exclusively arctic fox-variant rabies detected in arctic and red foxes, as well as dogs and wolves. The Alaska cases were limited to northern and western coastal areas. A model based on northern Canadian cases links rabies with human activity, the Arctic coast and its cold climate, and reasonably predicts cases in Alaska. In contrast, southern cases show an ecological niche that is characterized by warmer temperature characteristics and which is not restricted to coastlines. Our results indicate that the rabies cases in northern Canada occur in an area that is ecologically similar to those areas in Alaska that support maintenance of the rabies virus. This finding suggests that a circumpolar approach to rabies management would be appropriate for North America and possibly beyond. Further studies are needed to evaluate how the northern niche differs from, and connects with, southern rabies in this time of global climate change, in order to inform risk assessments and future management strategies.

NOT ALL SURVEILLANCE DATA ARE CREATED EQUAL - PRIORITIZING METHODS FOR ESTIMATION OF RABIES VIRUS ELIMINATION FROM WILDLIFE

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Wildlife diseases cause significant threats to humans, livestock, and wildlife. A necessary component of effective wildlife disease elimination programs is robust surveillance. Being able to distinguish between elimination and non-detection can be the difference between a successful elimination campaign and new epizootic events. We analyzed 10 years of raccoon rabies virus (RABV) surveillance data to estimate the detection probabilities from different types of surveillance samples (e.g., strange acting reports, road kill, trapped animals, nuisance animals) and evaluated the probability of raccoon RABV elimination in an enzootic zone with active oral rabies vaccination (ORV) management in progress using a dynamic occupancy approach adapted for multiple detection methods. We validated estimates from enhanced rabies surveillance data collected by the United States Department of Agriculture (USDA), Wildlife Services, National Rabies Management Program (NRMP) using positive cases from public health surveillance data reported to the Centers for Disease Control and Prevention. Using NRMP surveillance data, we found strange acting animals and animals found dead were twice as likely to be confirmed rabid compared to road-killed animals, three times as likely compared to trapped animals, and more than six times as likely to be confirmed rabid in comparison to nuisance animals. Our estimates of RABV occupancy were biased low compared to the public health positive data, and the bias was more pronounced in more rural areas. The probability of RABV elimination (probability of absence given non-detection) was negatively associated with the number of RABV cases in nearby areas, increased with time elapsed since the last RABV detection, and was lower in urban/suburban areas and higher in cultivated habitats and in the ORV management zone. This approach can be used compare different surveillance samples and examine relative biases as part of overall program evaluation, and is particularly important for opportunistic surveillance approaches.

VARIATION IN RACCOON HOME RANGE MOVEMENT DECREASES VACCINATION EFFECTIVENESS BY INCREASING THE SPATIAL SPREAD OF RABIES

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Host heterogeneity, including variation in susceptibility, infectiousness, and contact rates, have well established effects on disease dynamics such as driving explosive disease outbreaks. If heterogeneity in host movement promotes variation in an individual's capacity to contact or transmit disease to other hosts, theory predicts that increased host movement heterogeneity would cause higher spatial spread and incidence rates, potentially affecting vaccination effectiveness. To test this hypothesis, we developed a spatially-explicit, individual-based epidemiological model of raccoon rabies virus transmission to examine the effect of variation in raccoon home range movement on the spatial spread, persistence, and incidence of raccoon rabies. We also explored how raccoon movement variation influences population-level effectiveness of oral rabies vaccination strategies, including the proportion of the population that is vaccinated, the width of the vaccination zone where vaccines were delivered, and the frequency and timing of vaccination. We found that variability in raccoon movement increased the spatial spread rate of rabies by 1.2 - 5.2-fold compared to simulations in which all individuals within a simulation had a constant home range area and contact zone. Modest increases in movement variation led to large decreases in the relative effectiveness of a vaccination zone, such that more variable raccoon home ranges decreased oral vaccination effectiveness by 71% relative to less variable home ranges although the median home range size was similar across both groups of simulations. Our results suggest that heterogeneity in raccoon home range movement facilitates the spread and incidence of rabies, impeding the effectiveness of zone-based vaccination control measures. We argue that quantifying variation in host movement is critical for planning effective disease management strategies across broad landscapes. Our work supports a growing consensus about the importance of individual-level host heterogeneity on pathogen transmission, and provides insights for rabies management and elimination efforts using oral rabies vaccination targeting wildlife.

CHALLENGES AND INSIGHT FOR IMPROVING MANAGEMENT OF RACCOON RABIES IN DEVELOPED LANDSCAPES

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Effective wildlife rabies management is possible through oral rabies vaccination (ORV) targeting wild carnivore reservoir hosts. More than 10 million doses of oral vaccine are distributed annually in the US across broad landscapes in 17 states. Multi-year ORV efforts led to the elimination of canine rabies in coyotes in the US, near elimination of gray fox rabies in Texas, and has prevented appreciable spread of raccoon rabies variant (RRV) in the eastern US. Elimination of raccoon rabies is a national strategic goal requiring ORV in both rural and urban-suburban habitats. In 2016, a 24-member panel of rabies and wildlife experts was convened to provide consensus on the best approach for using ORV to eliminate RRV in the US. A comprehensive 30 year ORV strategy was proposed where ORV zones are established and moved sequentially in 3-5 year increments once local RRV elimination is achieved. Management of RRV in developed landscapes has proven challenging due in part to increased raccoon densities, smaller home ranges of target hosts, abundant anthropogenic food, non-target bait competition, habitat fragmentation and an inability to effectively bait available target habitats. Improved understanding of the ecology, movement and population demographics of rabies target and non-target species, enhanced vaccine bait performance and a refinement of ground and helicopter bait distribution strategies in urban environments is essential for RRV elimination. Research conducted by USDA Wildlife Services including ORV field trials, density studies, population ecology of raccoons, striped skunks and Virginia opossums and the use of Point of Interest GPS technology to enhance documentation of ground bait distribution has provided insight to improve wildlife rabies control in developed environments. Preliminary results from studies in these landscapes demonstrate fewer potential vaccine bait encounters for target species than expected, lower seroconversion rates compared to rural habitats and patchy vaccine bait distribution patterns.

SAFETY STUDIES IN THE SMALL INDIAN MONGOOSE (*HERPESTES AUROPUNCTATUS*) WITH THE ORAL RABIES VIRUS VACCINE STRAIN, SPBN GASGAS

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Oral vaccination of the small Indian mongoose (*Herpestes auropunctatus*) is presently under investigation in Puerto Rico. Besides field studies identifying a suitable bait and bait distribution strategy, experimental studies have identified an efficacious oral rabies virus vaccine candidate; SPBN GASGAS. Since, this vaccine strain is based on replication-competent viruses like all available oral rabies vaccines and vaccine baits are distributed unsupervised in the environment, stringent safety standards for such type of vaccines are required. The following safety studies in the target species were performed; overdose, repeated doses, dissemination and different routes of administration. It was shown that the construct was apathogenic, irrespective of dose and route of administration. The vaccine strain did not spread beyond the site of entry within the target species after direct oral instillation. Even when it was inoculated directly in the brain, it did not induce rabies infection. Hence, it was not possible to test reversion to virulence through serial passaging in the target species as requested. Instead, genetic stability including reversion to virulence was examined through 5 successive serial passages of the Master Seed Virus (MSV) in suckling mouse brain (SMB) and in the production cell line, BHK BSR Cl13. The consensus vaccine strain of the 5th passage, both in vivo and in vitro, showed 100% homology with the MSV and the 5th SMB-passage did not induce rabies when inoculated intracerebral in adult mice. Finally, all targeted genetic modifications in SPBN GASGAS remained unaltered after 5 serial passages (in vivo and in vitro). Thus, the vaccine strain SPBN GASGAS meets the safety requirements for live rabies virus vaccines in the target species, the small Indian mongoose.



**ORAL PRESENTATIONS
DIAGNOSTICS**

Moderators: CHRISTINE FEHLNER-GARDINER and
FERNANDO BELTRAN

COMPARISON OF PUBLISHED CONVENTIONAL AND REAL-TIME PCR ASSAYS FOR THE NEW OIE GUIDANCE THAT REQUIRES PAN-LYSSAVIRUS PCR FOR RABIES DIAGNOSTICS

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In 2018, World Organization for Animal Health (OIE) recommended pan-lyssavirus PCR assays as one of the primary diagnostic tests for rabies. PCR technology is widely available in the laboratories around the world. Incorporating PCR will improve the range of capacities and the overall quality of rabies diagnostics, especially in regions where specialized expertise and resources are limited. A pan-lyssavirus PCR assay detects highly diverged lyssaviruses in which the overall sequence similarity is as low as 60% between different species, therefore, increase its reliability of detecting rabies virus (RABV) which have the sequence similarities of 80% or higher. Conventional PCR and SYBR based real-time PCR use degenerated primers to achieve the pan-lyssavirus coverage, but degeneracy can produce non-specific amplification, which may necessitate additional DNA sequencing in order to confirm a result. Taqman based real-time PCR assays use probe(s) to improve specificity but are highly sensitive to the variations in the target sequences. Most published Taqman assays use multiple targeting sites and high level of degeneracy in probe sequences to overcome the sequence variations among RABV and other lyssaviruses. By comparing the probe sequences used in those assays against Genbank database, we identified those RABV and lyssavirus strains with 2 or more nucleotide differences between an assay's probe and its targets, which are predicted to render the assay less sensitive or to fail entirely at detection of the strains. This analysis suggests that an increasing of sequence degeneration for an assay's probe is not a solution for the pan-lyssavirus Taqman PCR design. The pan-lyssavirus Taqman assay LN34 uses less degeneration and instead relies on modified nucleotides for its probe to improve the assay's performance. LN34 assay is the only validated Taqman RT-PCR assay to detect all known RABV and lyssavirus species from both laboratory confirmation and in silico analysis.

DETECTION OF RABIES VIRUS ANTIGEN BY INDIRECT RAPID IMMUNOHISTOCHEMISTRY TEST IN EQUINES: COMPARATIVE STUDY WITH DIRECT FLUORESCENT ANTIBODY, MOUSE INOCULATION, ISOLATION IN CELL CULTURE AND REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION TESTS

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The techniques recommended by WHO for rabies diagnosis have high sensitivity and specificity, however, in equines sometimes the diagnosis is difficult by conventional methodologies. The objective of the study was to use the indirect rapid immunohistochemistry test (iRIT) for detection of rabies virus antigen in equines samples that presented discordant results by diagnostic techniques previously performed: direct fluorescent antibody (DFA) assay, mouse inoculation test (MIT), viral isolation in cell culture (VICC) and reverse transcription polymerase chain reaction (RT-PCR). During July 2014 until June 2016, 174 central nervous system (CNS) samples of equines were sent to the Instituto Pasteur, São Paulo, Brazil, of which 54 were diagnosed positive for rabies and 29/54 had discordant results among the techniques. The positivity by techniques was: 4/29 (13.8%) by DFA, 5/28 (17.9%) by VICC, 10/29 (34.5%) by MIT and 26/27 (96.3%) by RT-PCR. To perform the iRIT of these 29 samples, imprints of the cortex, hippocampus, cerebellum and brainstem were made in slides and fixed in 10% buffered formaldehyde. Hyperimmune serum against rabies virus produced in mouse (Instituto Evandro Chagas, Belém, Brazil) was used to detect rabies virus, followed by incubation with EnVision-HRP detection system. As result, we observed that the iRIT confirmed the rabies diagnostic in 18/29 (62.1%) samples analyzed, presenting a higher detection than the DFA, VICC and MIT, excepted for RT-PCR. However, freeze-thaw cycles of the samples may have interfered in the detection of viral antigen by iRIT. In relation to fragments, the brainstem presented a greater positivity, followed by the hippocampus, cortex and cerebellum. Our results provided evidence that iRIT contributes to the diagnosis of rabies in the equine species, and consequently to prophylaxis measures for contactant individuals and/or beaten by suspected animals. In addition, the study reinforces the importance of association of two or more techniques for better diagnostic accuracy.

CHARACTERIZATION OF ANTI-NUCLEOPROTEIN ANTIBODY FOR LYSSAVIRUS ANTIGEN DETECTION BY IMMUNOHISTOCHEMISTRY TEST

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Lyssaviruses are the causative agent for rabies. One of the primary diagnostic tests for confirming lyssavirus infections are antigen detection as a measure of infection, which refers to presence of lyssavirus encoded proteins in brain tissues of suspect animals. Although, the genome of lyssaviruses encodes five proteins, the antigen detection assays primarily focus on nucleoprotein (N protein). N protein is the most abundantly expressed protein after lyssavirus infection and accumulates in infected cells as inclusions. Direct fluorescent antibody (DFA) test, the most widely used method requires fresh or frozen brain tissues for N protein detection. In addition, the immunohistochemistry (IHC) test for rabies diagnosis has shown utility on formalin-fixed tissue for N protein detection and offers other advantages like; determine histopathological changes, simplify specimen storage and transport, minimize exposure to infectious agents and ability to perform retrospective studies. Sensitive and specific antibodies to the N protein are crucial for performance of these diagnostic assays. In this study, we characterized anti-N protein polyclonal antibodies (N pAb) raised in rabbits against recombinant, purified rabies virus (RABV) N protein produced in *Escherichia coli* by IHC test. The N pAb exhibited strong, specific reactivity against N protein expressed by diverse lyssaviruses in archived tissue samples. The sensitivity and specificity of N pAb and its utility in rabies diagnostics compared to mouse monoclonal or polyclonal antibodies generated upon immunization with RABV will be presented.

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Background: The Milwaukee Protocol has generated 18 survivors. Management and optimal outcome depend on systematic surveillance for rabies antibodies developing in blood and cerebrospinal fluid (CSF) by rabies reference laboratories that are often logistically inaccessible for rapid reporting of results. Delays are associated with poor outcomes. The commercial RAPINA lateral flow assay has been used for field epidemiology by detecting human serum antibodies to the rabies virus G glycoprotein. We tested RAPINA for quantifying human antibodies to rabies G glycoprotein in CSF and serum for bedside use.

Methods: Human serum or plasma and CSF with documented neutralizing antibodies to the rabies virus were serially diluted and tested using disposable RAPINA cartridges. Diluents included artificial CSF, phosphate-buffered saline (PBS), PBS-Tween and PBS-T 5% non-fat milk. Results were scored visually and evaluated after masking of samples.

Results: Serum samples from 5 rabies patients and CSF from 1 survivor were tested. Diluents, and plasma or serum, tested equivalently. The limit of sensitivity for RAPINA approximated 0.01 IU/ml in serum and CSF. Negative controls remained negative at all dilutions. Masked observers were 100% accurate.

Conclusions: Under pilot conditions, RAPINA cartridges performed accurately with high sensitivity and reproducibility. Testing required 1 hour, which can be contrasted to 3-10+ days for reporting under recent field conditions. Rabies antibodies must be quantified at thresholds of 0.3 IU/ml and 10 IU/ml during management of the Milwaukee Protocol. These thresholds correspond to serum and CSF dilutions of 1:30 and 1:1000 when applied to RAPINA cartridges. Using a reflex testing schema, 4-9 cartridges would be required per patient during 2 weeks of treatment using the MP. We will test RAPINA cartridges as a component of rabies treatment kits for on-site management of human rabies. RAPINA results should be confirmed by rabies reference laboratories, urgently rather than emergently.

GLYCOPROTEIN G RABIES VIRUS EXPRESSION IN INSECT CELLS FOR DIAGNOSTIC PURPOSE IN VACCINATED LLAMAS

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Presenter: Alexandra Marisa Targovnik

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Rabies is one of the principal zoonosis with worldwide distribution. The disease in llamas produces great economic losses in the productive system. The prevention against rabies virus (RABV) infection is done through vaccination with the inactivated virus. The level of protection can be assessed by determining the titre of neutralizing serum antibodies induced by RABV glycoprotein (RABV-G) in vaccinated individuals, which can be measured using an enzyme-linked immunosorbent assay. The aim of this work is to optimize a process for the production of the RABV-G ectodomain in Sfg cell. The recombinant baculovirus was constructed by introducing the sequence corresponding to the ectodomain region of RABV-G, under the polyhedrin promoter, fused to the viral signal peptide GP67 at its amino terminus and a 6-histidine tag at its carboxyl terminus. Despite the inefficient secretion of the RABV-G, it was possible to recover it from the cell lysate. Insect cells infected at multiplicity of infection of 0.5 at day 3 expressed 7 mg litre⁻¹. The recombinant RABV-G had a molecular mass of approximately 50 kDa according to SDS-PAGE analysis. PNGase F and Tunicamycin treatment confirmed that the protein is glycosylated. RABV-G was identified by LC-MS and was purified by metal ion affinity chromatography directly from the cell extract with a yield of 99% and a purity of 67%. Purified RABV-G was successfully used to detect specific antibodies in serum samples derived from rabies-vaccinated llamas. These results indicate that the recombinant RABV-G can be used as an antigen in the development of diagnostic kits.

La Rosa I, Presterá NE, Díaz Pérez PM, Panozo R, Caraballo DA, Tealdo MS, Beltrán FJ, Gury Dohmen FE.

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Rabies is a major public health problem in our country. It is a fatal encephalitis, caused by a highly neurotropic RNA virus, genus *Lyssavirus*, family *Rhabdoviridae*. Detection of rabies antigen in brain tissue by the Fluorescent antibody test (FAT) technique remains the gold standard for diagnosis. Also, a confirmatory test in which amplification of the virus is performed in a biological system is needed for a definitive rabies diagnosis. Traditionally, mouse inoculation test (MIT) has been used with high concordance. A disadvantage is that this technique involves the use of laboratory animals. Cell culture isolation test (RCIT) is an alternative that avoids this issue. The World Organization for Animal Health (OIE) recommends to test cell sensitivity for the virus variants circulating in the area. In this sense, our objective was to analyze the cell culture isolation of different variants found in Argentina from different species. Neuroblastoma cells were cultured in MEM 10 at 37°C in 5% CO₂ humidified atmosphere. Brain tissue homogenates were centrifuged. Supernatants were coincubated with cell suspension (2×10^5 cell/ml) and seeded in a 96 well plate and incubated for 96 hours. Cells were fixed and stained with FITC-antinucleocapsid antibody. Rabies variants were determined by monoclonal antibody panel and sequencing. During 2015-2018 a total of 95 FAT positive samples were isolated by RCIT from dogs (V1 and V2), cats (V1 and V4), cows (V3a), and insectivorous bats (V4, V6, *Eptesicus*, *Histiotus* and *Myotis*). These results indicate that the variants circulating more frequently in Argentine could be detected by the isolation test in neuroblastoma cells. This is of major importance to establish RCIT as confirmatory test and to encourage other laboratories in our country. It is notable that cell isolation is a useful tool not only for diagnosis but also for research.



**ORAL PRESENTATIONS
BAT RABIES**

Moderators: LUIS LECUONA and SUSANA RUSSO

IDENTIFICATION OF MUTATIONS IN THE RABIES VIRUS P GENE AFTER SUCCESSIVE PASSAGES IN MICE

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Rabies lyssavirus (RABV) is a virus with a single stranded RNA genome that encodes five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA dependent polymerase (L). Despite, RABV seems remarkably stable, differences among isolates from different natural reservoirs have been recognized for many years, suggesting adaptation of the viruses to some animal species. These are very peculiar since, in most species, the virus causes lethal infections and does not allow for co-evolution. Knowledge on the events that lead to adaptation of RABV to a particular host species is still scarce. In order to investigate such phenomenon, RABV strains adapted to different host species (IP964/06 from non-hematophagous bat *Eptesicus furinalis*; IP4005/06 from hematophagous bat *Desmodus rotundus*; CVS adapted to mice) were inoculated in mice for ten sequential passages. After each passage, the viruses were examined in its genetic profile. Total RNA was extracted and submitted to reverse transcription followed by polymerase chain reaction (RT-PCR) using primers targeting regions the whole phosphoprotein (P) gene. The amplicons were purified and sequenced. Sequences (nt 1514-2407) were edited, aligned and the putative amino acid (aa) sequences were deduced. Nonsynonymous nucleotide substitutions were identified in the P gene in IP964/06 from the non-hematophagous bat *Eptesicus furinalis* at aa position 222 (Isoleucine/Ile- Valine/Val) after the third passage in mice. This substitution was maintained over 10 passages. No substitutions were identified in IP4005/06 nor in CVS. No changes in the profiles of *in silico* restriction endonuclease analysis were detected with any of the three strains, despite the nonsynonymous substitution in isolate (IP964/06). Only three successive passages were necessary to induce such a mutation. Additional studies are necessary to investigate the significance of these findings in terms of adaptation of the virus to a new host species. Financial Support: Instituto Pasteur, FAPESP/ (2013/15760-0).

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Presenter: Diego Caraballo

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Bats are main reservoirs for Lyssavirus worldwide, constituting an important public health issue since they represent one of the main challenges in rabies control. Different rabies virus (RABV) variants are maintained by non-hematophagous bats in nature which are mainly species-specific. The correct identification of both bat species and RABV variant are essential in epidemiological surveillance to detect new variants as well as spillover events.

In this study, we implemented a molecular phylogenetic approach to identify bats at the species level. A total of 15 unknown specimens, belonging to two families, Vespertilionidae (*Myotis* spp.) and Molossidae (*Molossus* spp. and *Eumops* spp.), captured in the province of Santa Fe were studied. Genomic DNA was extracted from patagium or muscle samples. The complete mitochondrial cytochrome-b gene was amplified by PCR and sequenced using both primers. A Bayesian molecular phylogeny was obtained including unknown samples, plus 3 outgroups and 88 additional sequences representing all possible genera and allied species present in the sampling area, retrieved from Genbank.

Molecular analysis show that samples belong to three genera: *Myotis*, *Molossus*, and *Eumops*. The three *Eumops* samples were unambiguously identified as *E. bonariensis*. Five *Myotis* samples were identified as *M. nigricans*, but their possible ascription to *M. aff. diminutus* is discussed. The 7 remnant samples segregate into two subclades within *Molossus molossus*, but the possible relationship between one of these clades and *M. currentium* is examined.

This method proved to be useful for the identification of bat species when there is a reliable pre-existing classification. When such classification is absent, this approach combined with morphological data could serve to generate reference sequences to further identify samples remitted in the future.

AN UNUSUAL MORTALITY EVENT WITH EUROPEAN BAT LYSSAVIRUS TYPE-2: ENHANCED DIAGNOSIS THROUGH MOLECULAR TESTING

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The lyssaviruses constitute an important group of viruses capable of causing rabies. Whilst classical rabies virus causes 59,000 human deaths each year, only a handful of human deaths have been attributed to other lyssaviruses since their discovery over 50 years ago. Non-rabies lyssaviruses are generally associated with the infection of bats and novel members of the genus have been described at a steady rate over the last 20 years. Across Europe, several bat lyssaviruses have been described whilst classical bat rabies appears absent from the Old World chiroptera. European bat 2 lyssavirus (EBLV-2) was first detected in the UK in 1996. Until 2018, a total of 15 cases had been reported, all from Daubenton's bats, found either dead or with clinical disease. A single fatal case of EBLV-2 associated human rabies was reported in 2002 in a UK bat conservationist. However, in June-July 2018 an unusual mortality event was detected in a Daubenton's bat roost in East Sussex, England. Twelve bats were submitted to APHA for lyssavirus screening under the APHA passive surveillance scheme. The high mortality coincided with a heatwave in the UK. Many of the carcasses submitted were severely decomposed and were untestable using standard rabies diagnostic tests such as FAT and virus isolation. An initial FAT positive case in a testable bat led to a retrospective and more detailed investigation of the decomposed bats using SYBR real time RT-PCR. We subsequently detected EBLV-2 RNA in a further four Daubenton's bats from the site. The recent acceptance of rabies RT-PCR in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018 enables results from PCR assays to be officially reported. Further investigations into the basis for the high mortality and increased EBLV-2 detection are warranted.

VAMPIRE BAT BORNE PARALYTIC BOVINE RABIES AND LANDSCAPE STRUCTURE IN URUGUAY

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Bovine rabies transmitted by vampire bats was first confirmed in the Neotropics in 1911 and has since been detected across the distribution of vampire bats. However, Uruguay is an exception: it was free of bovine rabies until 2007, despite high cattle densities, the presence of vampire bats, and a strong surveillance system. All cases have detected since 2007 have been restricted to two foci of transmission. To explore why Uruguay was free of bovine rabies until recently, we reviewed the historic literature and reconstructed the conditions that would allow rabies invasion into Uruguay. We used available data on livestock abundance, distribution of vampire bats, and occurrence of rabies outbreaks, as well as environmental modifications, to assess support for four alternative hypotheses of viral invasion: host species movement, viral invasion, surveillance failure and ecological changes. Since the 1990s, Uruguay has experienced an increase of forest cover related to substitution of native grasslands for exotic, monospecific forestry for wood and cellulose production, coincident in space and time with the outbreaks. Using spatial autoregressive models and geographically weighted regression, we showed that the increase of grassland fragmentation by forestry ventures, together with the minimum temperature in the winter, explains the spatial distribution of outbreaks in the country. We propose that fragmentation increases connectivity of vampire bat colonies by increasing sharing of feeding areas, while temperature modulates their home range plasticity. Thus, vampire bats would increase connectivity in fragmented grasslands if they are in temperature-favorable areas. Increases in connectivity would promote a metapopulation dynamic that could explain persistence of the disease in the colonies. Our results suggest that land use planning might help to reduce fragmentation of grasslands and then reduce the risk of rabies transmission to livestock. This will be especially important in a context of increasing minimum temperatures in the winter.

BATS OF OCCURRENCE IN THE CERRADO AND AMAZONIA BIOMAS OF BRAZIL AND THE RELATIONSHIP WITH HUMAN RABIES

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Rabies is an acute infectious-contagious disease, characterized by symptoms of neurological pathology, which evolves to death within a few days. The socioeconomic losses caused by this disease, as well as the risks to public health, have required governments and breeders to take concrete actions to combat transmitters, but specifically to bats. In Maranhão, 31 cases of human rabies were recorded in 2005, in Pará there were more than 20 cases of human deaths from rabies transmitted by bats in 2004 and 2005. Costa et al. (2013) presented data collected in 2009 in northeastern Pará and found positive serology for rabies in more than 50% of bats of different species, this indicates viral circulation in this state. In Maranhão there is a record of positive cases of rabies in cattle transmitted by bat in the years of 2013 to 2015. In this study it was verified the occurrence of rabies virus in the bats of occurrence in the Cerrado and Amazonian biomes of Brazil, in the municipality of Caxias/MA (Cerrado) and in the municipalities of Turiaçu/MA, Cândido Mendes/MA, Godofredo Viana/MA, Carutapera/MA, Viseu/PA and Augusto Correa/PA (Amazônia), in the 2015/2016 biennium, the collected bats were taken to the CESC/UEMA laboratory where the brain was removed for diagnosis by the direct immunofluorescence technique (DFI). Twenty-six species belonging to five families were collected, the result was negative for all specimens analyzed independently of the species, therefore negativity for the species analyzed is an indication that these areas are under control, however, surveillance has to be maintained, because in the cerrado of Maranhão positive cases were recorded in cattle in the year 2015 and in Pará, in the Amazon biome, this year 14 suspected cases of human rabies with a confirmed case were reported.

RISK MODELING OF BAT-TRANSMITTED RABIES IN THE CARIBBEAN ISLANDS AND THE POTENTIAL FOR INTER-ISLAND MOVEMENTS OF BATS

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Rabies virus has persisted in the Americas for thousands of years, and although control efforts to decrease canine-transmitted rabies have been very effective over recent decades, there are still few effective interventions to control rabies spread by hematophagous and insectivorous bats. Rabies epidemiology and control measures vary significantly between Caribbean islands; additionally, studies on island-specific risks of bat-rabies have not been conducted on most islands and bat-rabies surveillance activities are largely absent in this region despite claims that many islands are historically 'rabies-free'. Data on bat rabies prevalence and distribution, as well as bat movements and movement potential are limited for this region, which constrains traditional risk mapping techniques. We used the analytic hierarchy process (AHP) to create pairwise comparison values among five risk factors determined by bat-rabies subject matter experts. These values were aggregated into a matrix to calculate weights for each risk factor, these weights were used to assign risk values in a geospatial analysis (higher values indicate higher risk that rabies virus is present in endemic bat species). Risk values ranged from 8.52 (Trinidad) to 1.27 (Saba, St. Barthelemy, and St. Eustatius). All four countries that currently have or have had a history of bat-rabies were ranked highest (Trinidad, Grenada, Cuba, Dominican Republic), as well as Haiti which shares a landmass with the Dominican Republic. The five highest risk 'rabies-free' countries included Aruba, St. Vincent & the Grenadines, Puerto Rico, Jamaica, and Dominica. A literature review conducted in this study highlights the inter-island movement potential of bats, as well as areas of high risk for bat-associated rabies in the Caribbean islands, and demonstrates a need for further surveillance efforts in historically 'rabies-free' island nations.



POSTER PRESENTATIONS
MONDAY 29 OCTOBER 15:00 - 16:30

COMPARISON OF STREET RABIES VIRUS INFECTIVITY IN DIFFERENT CELL LINEAGES

Nayara Ugeda Silva, Natalia Langenfeld Fuoco, Orlando Garcia Ribeiro, Fernanda Guedes Luiz, Sandriana dos Ramos Silva, Elaine Raniero Fernandes, Iana Suly Santos Katz
Instituto Pasteur.

Presenter: Sandriana Dos Ramos Silva

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Rabies is caused by a neurotropic virus that is transmissible to all mammals. Rabies virus (RABV) isolated from different reservoirs have distinct biological properties, which leads to differences in pathogenicity and virulence to the host. It is known that RABV strains from bats and canines could exhibit differences in their glycoproteins, thus using different receptors in order to infect cells. Here, we evaluated and compared the infectivity of RABV from vampire bat (V-3) and canines (V-2) in different cell lineages. For this, the virus growth curves, at 0.05 and 5 MOI, and kinetics of virus internalization of RABV variants isolated from V-3 and V-2 were performed in mouse neuroblastoma (NA), baby hamster kidney (BHK-21) and Monkey African Green kidney (Vero) cells. We verified that V-2 sample had higher replication rate at 96 h ($p < 0.001$) and delayed internalization in BHK-21 and Vero cell than V-3 isolated. However, at 96 hours, the replication rate of V-3 was significantly higher ($p < 0.001$) and presented a lower rate of virus uptake in NA when compared with V-2 sample. Our data demonstrated that different RABV strains have distinct capacity of replication and internalization, which is dependent on the cell lineages used. These results together support the hypothesis that dog and bat viruses use different cell-surface molecules for entry, which it could interfere in the diseases. The data obtained in this study can contribute to a better understanding of the virulence of the RABV maintained in different reservoirs in Brazil.

BRAIN GENE EXPRESSION PROFILES OF VARIANT 2 AND 3 STREET RABIES VIRUS INFECTED MICE

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Presenter: Jane Megid

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Rabies pathogenesis has been studied in many different aspects but a precise understanding of the mechanisms involved in this disease was not reached. Rabies virus strains differ in the level of inflammation, apoptosis and neural degeneration specially when 'street' rabies virus is compared to 'fixed' virus. The aim of this study was to evaluate the gene expression profile in brains of mice infected with street rabies viruses – dog variant (V2) and vampire bat variant 3 (V3). A total of 16 (8 animals per variant) C57/BL6 mice, SPF, female, 4–6 weeks-old, were inoculated via intracerebral route with 30uL of rabies virus (LD50 10-6,66); 8 mice were left as negative controls. Inoculated animals were observed and weighed daily in order to determine the onset of clinical signs. Whole brain was collected immediately after death for microarray analysis. Extraction of total RNA was performed with a commercial kit and gene expression analysis was performed using the GeneChip® Mouse Gene 2.0 ST Array from Affymetrix®. Gene enrichment, canonical pathways and gene ontology were determined using NIPA (<https://github.com/ADAC-UoN/NIPA>). For V2, analysis revealed 762 genes were up-regulated and 291 down-regulated and for V3, 554 genes were up-regulated and 75 down-regulated. Common pathways were identified between both variants including antigen processing, MyD88 dependent toll-like receptor signalling, NOD-like receptor signalling, TNF and TLR signalling pathways, interferon beta production, differentiation of dendritic cells, increase of chemokines receptors and type II hypersensitivity. When DEGs from V3 and V2 were compared, a total of 25 genes were differently expressed; 3 genes up-regulated and 22 genes down-regulated. Nine main pathways were associated with these genes; being five related to the cell membrane, three associated with protein degradation, location and inhibition of protein interaction via ubiquitin activity and one related to receptor activity.

**Caio Vinicius Botelho Brito, Lavinia Dias Tavares, Érika Dayane Leal Rodrigues,
Fernanda Monik Silva Martins, André Luís De Sousa Nogueira Lima,
José Antônio Picanço Diniz Junior, Livia Medeiros Neves Casseb**
Evandro Chagas Institute, Secretary of health surveillance, Ministry of health

Presenter: Livia Medeiros Neves Casseb

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Human rabies is a very lethal viral encephalitis, being held responsible for approximately 59,000 deadly cases per year. The pathogenic agent is a RNA virus, the Rabies lyssavirus (RABV) in the genus Lyssavirus, mostly transmitted by the bite of other infected mammals. The infection first affects the myocytes and then the peripheral and central nervous system (committing the motor and sensor neurons). The onset of symptoms is after 20-90 days post RABV exposure with two clinical presentations: encephalitic (furious) and paralytic (dumb). Moreover, the RABV infection remains without a specific and efficient therapy established. Due to RABV infection's epidemiologic relevance, this study objectifies to evaluate the clinical presentation of mice infected with RABV and submitted to treatment protocols. It is an experimental study with the approval by the Ethics Committee for Animal Research. The research used 16 isogenic female mice, on whom was inoculated the RABV variant 3. The meticulous clinical observation of the animals started in the 1st day post-infection (DPI), being divided in four moments and with evidence of clinical signs to the 18th DPI until the 28th DPI. The evaluation clinical parameters were disposed on protocols formulated by the researchers and were divided in general exam, assessment inside the box, on open field, of the tail suspension and during the supination contention. Most animals did not present symptoms during the experimentation's progress, even in the presence of positive direct immunofluorescence for RABV. The most common clinical presentation was piloerection without additional symptoms, opposing to the classical form of RABV in mice that holds piloerection, walking difficulty, absence of biped posture, absence of response on back paws, body position with spasticity in lower limbs, no spontaneous activity, locomotor activity of 3 to 4 cm, no apprehension on wire test, no pelvis suspension and bilateral areflexia. Furthermore, two mice in the infection control group presented symptoms suggestive of transversal myelitis (commitment on the level of third lumbar vertebra, areflexia of lower limbs, absence of tail suspension and fecal and urinary incontinence). Therefore, the conclusion is that the clinical parameter for rabies on animal models is not appropriate to evaluate the infection evolution, using as basis the direct immunofluorescence.

Stein, Michal M.D; Meidler, Roberto Ph.D; Bergman, Garrett E, M.D; Costa, Peter; Alma Levy Ph.D
Michal Stein
Kamada

Presenter: Shavit Belaved

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Rabies is one of the most deadly, and most preventable infectious diseases responsible for an estimated 59,000 human deaths every year¹. Post-exposure prophylaxis (PEP) is essentially 100% effective in preventing human rabies after exposure to a rabid animal. According to WHO guidelines, PEP consists of thorough wound washing, adequate wound infiltration with rabies immune globulin (RIG) and a series of rabies vaccine doses². Barriers to implementing proper rabies PEP after exposure include RIG and vaccine availability and supply, cold chain requirements, product quality, shelf life, and others. Since RIG neutralizes the rabies virus locally in tissues, before the subject's immune system produces rabies virus neutralizing antibodies in response to vaccination, it constitutes a critical part of rabies PEP in severe (grade III) cases of exposure. On a global level, cold chain requirements are one of the main barriers to RIG availability. Kamada-HRIG, Rabies Immunoglobulin (Human) was approved by the US FDA in August 2017. The product may be kept in room temperature ($\leq 77^\circ$ F) up to 30 days, which may enable its availability for immediate usage upon need. Following storage at room temperature the product should be either used or discarded. The extended temperature stability may be beneficial for RIG use in remote areas, field-based medical applications such as wilderness medicine and armed forces operations. Room temperature storage may also help reduce pharmaceutical economic impact and costs by decreasing the likelihood for waste due to temperature excursion. Room temperature stability of Kamada-HRIG is likely attributable to its high-purification production process which eliminates proteases that could degrade the product, facilitated by a low pH to reduce aggregation and further limit proteolytic activity.

Due to its extended stability at room temperature up to 77F, Kamada-HRIG should be evaluated for use in rabies endemic locations wherever proper refrigeration cannot be assured.

References:

1. Hampson, K et al. Estimating the global burden of endemic canine rabies. PLoS neglected tropical diseases, 2015; 9(4), p.e0003709.
2. WHO Guide for Post Exposure Prophylaxis | www.who.int/rabies/human/postexp/en

PROFILE OF ANIMAL BITE VICTIMS ATTENDING THE C.G. DURAND HOSPITAL ANTI- RABIES CENTRE OF BUENOS AIRES CITY IN ARGENTINA (2014-2017)

Langan ME, Cosido S, Kitos E, Daghlian MR, Alvarez LV, Acrich G, Avello R, Tissera ML, Erviti A, Lee F, Bruno ME.

Chief of Promotion and Prevention Division. Carlos G. Durand General Hospital

Presenter: Miriam Bruno

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In Buenos Aires City, the only Centre for Human Rabies Prophylaxis which belongs to the Public Health Care System is in the C.G. Durand Hospital. All vaccines and rabies immune-globulin were supplied by the National Ministry of Health.

Buenos Aires City (BAC) has a population of 3 million people and from January 2014 until December 2017 an average of 8.500 persons a year, who had been bitten by animals were assessed and treated at the Centre. Seventy five percent of these patients were residents of BAC, resulting in annual incidence rate of bitten of 212 /100.000 inhabitants. Children younger than 15 represented 35% of the consultations and 5% were admitted. The medium age of patients was 5 years old and the highest incidence of bitten children was in the 2 years old group. Dogs were involved in 80% of visits, but 95% in <15 years of age and 70% were home pet dogs.

From January 2014 until December 2017 the Durand Hospital Anti-Rabies Centre applied an annual average of 23.600 doses of human rabies cell-culture vaccines for post-exposure prophylaxis according with Essen schedule (five-dose course). However in 2017, due to a shortage in supply, 17.800 doses were applied in immunocompetent people using a modified schedule (four-dose course).

From 2014-2017 three hundred and thirteen patients at the Durand Hospital Anti- Rabid Centre required rabies immune-globulin to treat wild animal bites, mostly bats and coatis. This entailed 1.377 vials of this biological which were always combined with active immunization.

Domestic animals' bites are a significant cause of visits to the C.G. Durand Hospital. Having the possibility of studying our patient population and analyzing the circumstances in which these bite injuries occur allow us to develop strategies for prevention of these injuries as well as to optimize the use of our resources.

Lima, FMG; Moura, FBP; Chaves, CS; Maia, KM; Lima, MFC; Rodrigues, VC
Secretaria Estadual da Saúde do Ceará

Presenter: Fabíola Maria de Girão Lima
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Rabies is an acute viral infectious disease that affects mammals, including humans, and is characterized as a progressive encephalitis with approximately 100% lethality. In Brazil from 2010 to 2017, 25 cases of human rabies were recorded, eight of them transmitted by *Desmodus rotundus* bats species*. Therefore, the present work aims to describe a case of human rabies, transmitted by *Desmodus rotundus*, in 2016, in Ceará State. The event took place in a rural area of Iracema, a small municipality in the state. A visit was made on the spot for epidemiological investigation; the patient's wife reported that in early September, her husband (A. N. S., 37), asleep, suffered a bat attack but did not want to seek medical attention. So, 15 days later he began to present nausea, weak legs, migraine, thigh pain, but continued without seeking care. In mid-October he she began to feel a pain "ripping" in her legs and "fingers burned." Then, he sought municipal hospital, remaining there hospitalized with worsening of the clinical picture. After a few days, the doctor inquired about the occurrence of some animal aggression, and then bat aggression was reported. The patient was transferred on 10/20/2016 to the reference hospital in state capital with paralysis of lower limbs and anuri. Then, PCR rabies of the hair follicle was confirmed. Subsequently, the human rabies treatment protocol, based on the induction of deep coma. The use of antivirals and other specific drugs was used. Without success, the patient evolved to death on 11/11/2016. It is inferred that the patient was not aware of rabies because he did not seek prophylactic treatment, a decisive factor for the evolution of the disease. Such an episode induced the municipality to rethink and evaluate its actions surveillance and prevention of human and animal rabies.

BEGINNING THE PROCESS OF UPDATING THE ADVISORY COMMITTEE ON IMMUNIZATION PRACTICE GUIDELINES FOR RABIES--UNITED STATES 2018

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Background: The Advisory Committee on Immunization Practices (ACIP) is comprised of experts who develop guidance for safe use of vaccines among civilian populations in the United States (U.S.). ACIP guidelines for rabies were comprehensively updated in 2008, followed by an update to the post-exposure prophylaxis schedule in 2010; since then, data with direct relevance to the vaccination schedules and serologic monitoring recommendations have been published. In response to this evidence, the World Health Organization (WHO) has updated global recommendations.

Methods: The Centers for Disease Control and Prevention's Office of the Associate Director of Science sets rigorous standards and expectations for guideline development. Early steps include identifying working group members who will review and deliberate questions, determining the scope of the update, and outlining activities that will facilitate effective discussion. Working group members and ACIP committee members are selected for their experience and expertise in immunobiologics, vaccine research, vaccine efficacy and safety, and consumer perspectives; working group members will deliberate topics before they are presented to committee members for formal vote.

Results: We identified preliminary stakeholders in clinical practice and public health who can represent their associations as working group members; these include national public health organizations comprised of veterinarians or local health officials, and clinical experts in pediatrics, emergency medicine, and pharmacology. The scope of the guidelines will include vaccination schedules, safety and effectiveness of new and existing biologics, cost-effectiveness of vaccination schedules, and location of immunoglobulin administration. We will determine the epidemiology and burden of rabies exposures and post-exposure prophylaxis in the U.S. and perform systematic reviews and other analyses to fill existing literature gaps. Serologic monitoring recommendations and rabies risk within the U.S. will be evaluated and WHO recommendations reviewed.

Conclusions: ACIP guidelines for rabies will be updated based on established scientific standards to provide new evidence-based recommendations.

DEVELOPMENT OF AN INDIRECT ELISA BASED ON PURIFIED, INACTIVATED RABIES VIRUS AS A FIRST STEP TO EVALUATE IMMUNE RESPONSE INDUCED BY RECOMBINANT ANTI-RABIES VACCINES

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Preexposure rabies vaccination is recommended for susceptible animals in areas where the disease is endemic. In our laboratory we develop pox and adenovirus-vectored vaccines to prevent paralytic rabies in bovines. In order to rapidly evaluate the induction of specific antibodies by our recombinant vectors prior to test them in efficacy trials, a simple method is required. Thus, the aim of this work was the development of an indirect ELISA to detect anti-rabies antibodies in animals inoculated with the recombinant immunogens.

ELISA was performed by immobilization of the antigen in microplates of 96 wells. Several parameters were evaluated in order to improve the specific signal with minimal background: 1) amount and type of antigen bound on the plates; 2) period of time for antigen binding; 3) blocking solutions; 4) dilution of the positive sera.

Various antigens were evaluated at different quantities per well: Rabipur vaccine (1-3 ug), concentrated inactivated rabies virus (RVi) (50-800 ng), sucrose-gradient purified RVi (0,4-3,2 ug) or extracts from cells infected with recombinant baculovirus (Bv) expressing rabies glycoprotein (50-600 ng of RG). The antigen binding was accomplished for 1 h at 37°C or 16 h at 4°C. The blocking solutions used were: 3 or 5 % skimmed-milk and 1% PVP solution. The negative and positive mice sera were diluted 1/25; 1/50 or 1/100 in blocking solution. As a second antibody a HRP-anti mouse antibody was used. After 30 min of incubation with ABTS substrate solution, the optical density was measured at 405 nm.

Best results were obtained using 2 ug/well of purified RVi bound 16 h at 4°C, PVP solution for blocking and sera diluted 1/50. Finally, we confirmed by ELISA that MVA vector expressing rabies glycoprotein induces in mice a specific immune response significantly different from those animals inoculated with non-recombinant MVA.

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In China and other parts of the world, about 5% of the human rabies cases are caused by cats. Rabies vaccination in cats is a must in all developed countries. However, only inactivated rabies vaccine is used in practice. In this study, the gI/E region of a felid herpesvirus 1 (FHV-1) isolate was deleted by homologous recombination and replaced by an expression cassette of the glycoprotein of rabies virus strain BD06. The expression of the glycoprotein was proved on cells by immunofluorescent assay (IFA). The cats were intranasally inoculated for once with 1ml containing 105.6 TCID₅₀ of the recombinant virus. The neutralizing antibody collected every two weeks for 6 months, and virus challenge trials was performed at the end of observation. The results showed that all vaccinated cats produced antibodies to both the FHV-1 and rabies virus (RABV). The rabies viral neutralizing antibody (VNA) reached a protective level since 14 days post inoculation (dpi) and mounted to a high level with about 30.07±10.23 IU/ml on 28 dpi, and last at least six months more. All the cats present no clinical signs of FHV-1 infection and survived the challenge of a BD06, comparing with a severe rhino tracheitis in all cats and 100% rabies death in the controls. To our knowledge, this is the first report of a herpesvirus-based recombinant vaccine effective in stimulating a protection against both FHV-1 and RABV infection.

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Presenter: Romina Scian

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The gold-standard method for determining the potency of rabies vaccines is a mice challenge test referred to as "NIH". In this procedure mice are vaccinated and challenged intracerebrally with rabies virus. The relative potency of the test vaccine is calculated on the basis of the survival rate compared with a reference vaccine. The NIH has been highly criticized for its poor precision and significant variability, and is questionable for ethical and practical reasons, considering the high number of animals involved. In 2013 the European Pharmacopoeia included a serological assay, for batch potency testing as an alternative to the mouse challenge test. This assay was validated at the Paul-Ehrlich Institute and demonstrated to be highly reproducible. It involves the immunization of a reduced number of mice, and the sera testing for rabies virus neutralizing antibodies using a rapid fluorescent focus inhibition test. A vaccine meets the potency requirement if the titre induced is not less than the titre yielded by a reference with proven potency.

In line with the 3R-concept of reducing, refining and replacing animal tests, Biogenesis Bagó acquired the necessary equipment and conducted several assays for the in-house adoption and verification of the serological method. The viral strain CVS used for the animal challenge test was adapted to the infection of BHK-21 cell line, and a viral stock was generated for the virus neutralization assay. The potency of 15 vaccine batches were analyzed by both NIH and the alternative method. The serological assay could be reproduced satisfactorily in our company, and the technic proved to be practical and fast. Its implementation could result in a reduction of animal numbers by almost 85%.

Additionally, Biogenesis Bagó is working on other in vitro methodologies for quantification of viral antigens by chromatography technics, which would contribute to reduce the use of animals.

QUALITY CONTROL OF RABIES VACCINES

Lic. Susana Russo; M.V. L. Novaro, Aux. Lab. G. Centurión

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The Rabies Department of SENASA, the National Veterinary Service of Argentina, since 1983 has a legal instrument that settles quality control norms for Rabies Veterinary Vaccines.

- Informs the dossiers of Veterinary Rabies Vaccines
- Controls 100% of the commercial national and imported lots

The following tests are performed to evaluate the biological products prior to licensing:

- Safety Control
- Sterility Control
- N.I.H. Potency Test
- Biologic Assay
- General Security
- Stability: Accelerated Thermal Degradation

Veterinary Antirabic Vaccines controlled during 2016, 2017 y 2018 (included until August) with the National Reference Vaccine DILAB PV-BHK-4.

Results:

- All the vaccines are inactivated and produced in cell cultures.
- Safety: Satisfactory
- Sterility: Satisfactory
- Monodose vaccines released to the market was 99% of the dose presented and 87.8% of the lots presented in this period and the multi-dose vaccines were 95.65% and 87.9%, respectively.

THE “CAOMINHADA” IN THE FIGHT AGAINST THE RAGE IN SÃO MIGUEL, SÃO PAULO

João Gabriel Zerba Corrêa, Wernner Santos Garcia, Edimar Alves de Azevedo, Fabio Junior de Oliveira Ferreira, Karen Fernanda de Freitas Garilho, Gisele Pereira Gonçalves, Claudia Pereira dos Santos Martinez

Presenter: Wernner Santos Garcia

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Rabies has been described at least 4 millennia ago and is one of the earliest known zoonoses transmitted by animal bites, such as dogs and cats. Due to its lethal evolution, the high number of cases in humans as well as people undergoing treatment every year, rabies remains a worldwide public health problem. In São Paulo alone, there were 26,016 bite cases in 2016. R \$ 300.00 (US \$ 95.75) is spent on treatment. Therefore, in September 2016, it was decided to create an event with actions, along with the dissemination of the services offered, by the Health Surveillance Supervision (SUVIS) of São Miguel Paulista, for orientation of rabies and control of bite cases of Animals. In this event, guidelines were given on biting dogs and cats (how to avoid biting, procedures at the time of bite, which equipment to seek care and what treatment to perform) and on bats (presentation of bat types and how to proceed Finding an animal), carrying out anti-rabies vaccination of the animals, conducting registration and referral for sterilization, and a walk with the animals, called “Cãominhada”, with medals for all participants. It counted with the participation of 500 people, and made 200 registrations and referrals for sterilization and 300 anti-rabies vaccinations. It was found that three months after the event there was a 46% decrease in bite cases in the region. This shows an economy for the public coffers, both in terms of treatment costs and the risk of spreading rabies. The event was also part of the World Rabies Day program of the Global Alliance for Rabies Control. It should be noted that due to the success for the year 2017, the second event is scheduled to take place.

CAOMINHAR - INTEGRATE, GUIDE AND PREVENT: SÃO MIGUEL PAULISTA IN THE RAGE COMBAT

João Gabriel Zerba Corrêa, Wernner Santos Garcia, Edimar Alves de Azevedo, Fabio Junior de Oliveira Ferreira, Karen Fernanda de Freitas Garilho, Gisele Pereira Gonçalves, Claudia Pereira dos Santos Martinez

Presenter: Wernner Santos Garcia
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Rabies has been described as one of the earliest known lethal zoonoses. In Brazil from 1990 to 2009, 574 cases of human rabies were registered, with only 26,016 cases of biting in São Paulo in 2016. Until 2003, the main transmitter was the dog and, since 2004, the bat. Nowadays, approximately R \$ 300.00 (approximately US \$ 77.60) in treatments are spent on bite patients. It was then decided to create actions to cover vaccination, education and elimination, together with the dissemination of the services offered by the Surveillance Unit, always focusing on the reduction of bite cases in the territory. For these actions, on September 16, 2017 was held the event "2 ° Caominhar, integrate, guide and prevent: São Miguel Paulista in the fight against rabies," with the assistance of animal protection NGOs and the faculty of veterinary medicine. In the action we obtained approximately 123 vaccinated animals, 86 registered animals (R.G.A.), including for future sterilizations, 250 veterinary guidelines, 150 distributed booklets of bite prevention for children and 800 participants. It was found that three months after the event there was a 30% decrease in bite cases in the region. This shows an economy for the public coffers, both in terms of treatment costs and the reduction of risks of rabies, and the request of the residents to hold the third event, being "important in the calendar of the region" and is already scheduled for 2018 "The action was also awarded at the Global Rabies Control World Rabies Day MSD Award event, ranking 2nd in 2016 and 1st place in 2017.

COST EFFECTIVENESS ANALYSIS OF VARIOUS CANINE RABIES VACCINATION SCENARIOS IN SUCHITEPEQUEZ, GUATEMALA

Seonghye Jeon, Julie Cleaton, Emily B. Kahn, Martin I. Meltzer, Emily G. Pieracci, Jesse Blanton, Ryan Wallace and David Moran

Presenter: Seonghye Jeon

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In Guatemala, the average canine rabies vaccination rate is approximately 35%, and there has been at least one human rabies deaths annually in the past five years. We evaluated the cost-effectiveness of 4 potential rabies control strategies for Suchitepéquez, Guatemala.

We used RabiesEcon, a deterministic mathematical model to compare the following strategies:

- a. No vaccination: Permanently discontinue the vaccination program and only administer human post-exposure prophylaxis (PEP).
- b. Stop-and-restart: Annually vaccinate 35% of dogs; when human deaths decrease to below 5 per year, stop; resume the program when human deaths rise to 5 or more per year.
- c. Constant (current strategy): Annually vaccinate 35% of dogs.
- d. Elimination effort: Annually vaccinate 70% of dogs until the model estimates canine rabies elimination. Post elimination maintenance operation will then be defined by the user.

As a sensitivity analysis, we varied the proportion of exposed humans receiving PEP (10% - 99%) and the number of dogs infected per infectious dog (1.2 -1.8). Finally, we calculated, using the government perspective, the costs and health benefits of each strategy over a 30-year period.

The 'elimination effort' had the lowest average cost per human death averted of 8,362 USD (range: 6,264 - 9,959 USD) in a 30-year period. With this option, endemic canine rabies ceased by year 3, and an additional 12 years of vaccination ensured no further cases, assuming no risk of re-introduction. The other 2 dog vaccination strategies had similar short-term cost-effectiveness, however, canine rabies remained endemic after 30 years of campaigns. The 'no vaccination' strategy cost 13,865 to 35,947 USD per human death averted, and had no impact on the number of rabid dogs.

In Suchitepéquez, Guatemala, canine rabies can be eliminated in approximately 15 years by vaccinating 70% of dogs. The estimates will aid the Ministry of Health in implementing such plans.

RABIES ANTIBODY LEVELS IN DIFFERENT GROUPS OF PETS FOLLOWING VACCINATION

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Rabies serology testing as a check of immunization is of interest to both those traveling with their animal companions and those uneasy about perceived over-vaccination of their pets. The Kansas State University Rabies Laboratory, an EU/OIE (World Organization for Animal Health) approved laboratory, provides serological rabies testing for pet travel purposes (since 2002) by two recognized testing methods: Fluorescent Antibody Virus Neutralization (FAVN) and Rapid Fluorescent Focus Inhibition Test (RFFIT). In 2015, a modified RFFIT assay was developed that allows for a cost effective way to measure antibody levels in dogs and cats whose owners have concerns about vaccine booster effects on health of their pet. Large number of samples tested for both these groups has allowed cumulative data for analysis of rabies antibody levels in companion animals. Though policies vary between quarantine stations of rabies-free destinations, a majority requires a minimum rabies antibody level ≥ 0.50 IU/mL as a part of their assurance of adequate vaccination of an animal. Due to these requirements, companion animals with an owner's intent to travel are normally well (multiple vaccinations) and/or recently vaccinated. Analysis of dog samples in the dataset identified a difference in percent of dogs with results below 0.50 IU/mL (low responders) for dogs traveling to rabies-free areas and dogs tested for immune status only: 4.5% and 16.1%, respectively. Thus, indicating a positive effect of intentional timing of vaccination and blood draw on the measured antibody response to rabies. In addition, data analysis for effect of age, size, and breed on antibody response reinforced previously demonstrated increased incidence of results below 0.50 IU/mL in young dogs. Moreover, while size had slight effect on the antibody level achieved, this association was not absolute since the groups of the highest and lowest incidence of low responders included small, medium and large breeds.

CONTROL DE FOCO RÁBICO A TRAVÉS DEL USO DE UNA APLICACIÓN MÓVIL

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En México se han fortalecido las acciones de prevención y control en el reservorio lo que ha permitido mantener al país sin casos de rabia humana. Este año en el Estado de Nuevo León, con apoyo de la Secretaría de Salud Federal, se implementó el uso de una aplicación móvil para medir el porcentaje de cobertura de vacunación en el Estado, y se utilizó esta herramienta para documentar su uso para medir el impacto de las actividades, en tiempo real, realizadas en un foco rábico. Se visitaron un total de 941 viviendas, de las cuales el 41.02% fueron encuestas, 40.70% se encontraban ausentes, 10.20% renuentes, 8.08% deshabitadas; se encontraron un total de 283 mascotas, el 85.87% fueron perros y el 14.13% gatos, de las cuales el 56.69% refirieron que les habían regalado las mascotas, el 16.54% habían nacido en el domicilio, el 9.45% fueron comprados y 14.96% habían sido recogidos de la calle. Solamente un 60.50% de la población animal presentaba esquemas de vacunación vigente. El uso de esta aplicación nos permitió contar con la información de manera oportuna permitiéndonos analizar y modificar nuestras estrategias de actividades para el foco rábico, detectar áreas de oportunidad en el personal como lo son: establecer un grupo de trabajo fijo de trabajo para los focos rábicos ya que la mayoría del personal era rotativo lo que hacía necesario que cada dos días se tuviera que capacitar al personal en la utilización de la aplicación y como factores determinantes de la localidad se encontró que la mayoría de la gente salía de sus domicilios a trabajar desde muy temprano lo que dificultaban la realización de la aplicación de la encuesta y la vacunación de los animales, teniendo que cambiar de estrategias para poder realizar los barridos en horario vespertino y fines de semana.

INTERVENTIONS FOR RABIES CONTROL OUTBREAKS BY THE NATIONAL PROGRAM OF ZOOSES CONTROL AND RABIES NATIONAL CENTER IN PARAGUAY (2017-2018)

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Rabies is a zoonotic viral disease, caused by a virus from the Lyssavirus genus of the Rhabdoviridae family, which infects mammals and causes fatal encephalitis. The Paraguayan Program for Rabies Control in herbivores aims to prevent the disease in cattle by focusing on the control of vampire bats (*Desmodus rotundus*), strategic vaccination and epidemiological surveillance of animal's rabies outbreaks cases based in the vaccination of human, dogs and cats exposed to the virus. Veterinary immunization is mostly provided by the Public Veterinary Services in coordination with the local municipalities; private veterinary services also provide vaccination. The last case of human rabies in Paraguay was in 2004. The aim of this study was to show the interventions for rabies control outbreaks occurred from January 2017 to August 2018 in Paraguay. There were notified by the National Service of Quality and Animal Health (SENACSA), the Rabies National Center and the Veterinary Diagnostic Laboratory (CEDIVEP), 87 cases of animal's rabies (85 cattle, 1 equine and 1 vampire bat). There were vaccinated with the Verorab® Sanofis®, France, vaccine against rabies in humans, 430 persons that were exposed to the virus (farmers, animal's owners), and 6072 dogs and cats living in a ratio of 5 kilometers around the animal's rabies case were immunized with the Rai-vet®, Biovet®, Brazil veterinary vaccine against rabies. Implementation of control measures for rabies we recommend. Aggressive interventions that include regular animal's vaccination campaigns for farmers and dogs and cat's owners, improvement in vaccines provision for humans and animals, accompanied by regular campaigns of education in communities and sanitary professionals in public health centers for appropriate prevention and control strategies against rabies were carried out.

THE HISTORY OF RABIES IN DOGS AND CATS IN THE STATE OF PERNAMBUCO, NORTHEASTERN BRAZIL: FROM THE SENDING OF SAMPLES

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Didactically, the cycle of rabies is divided into urban, wild, aerial, and rural. These cycles do not occur in isolation, and there are reports of transmission to humans via all of them. Urban rabies has dogs and cats as main actors, however, due to the efficiency of vaccination campaigns in Brazil, it has been virtually eradicated, and the canine variant of the virus is no longer found. The occurrence of rabid dogs and cats in the country takes place by contact with other variants. Despite the monitoring, in June 2017, a woman died of rabies transmitted by a cat in the city of Recife. This work surveyed the samples from dogs and cats sent by the state for rabies analysis, making a profile of the disease over the years. For this, the inventory was recorded in the LACEN sample collection records between 1975 and 2017, separating the samples sent by regions (Metropolitana, Zona da Mata, Agreste, Sertão do São Francisco, and Sertão). Thirty-eight thousand, three hundred and eighty-two (38,382) samples were sent (32,787 dogs and 5,595 cats), with the prevalence of dogs in all regions. There were 2,232 and 211 positive samples for dogs and cats, respectively, with positivity for dogs and cats in all regions. Only from 2012 to 2015 there was no positivity for dogs, and in eight of the years analyzed for cats. The first and last positive cases in both dogs and cats occurred in 1975 and 2017. The number of positive samples per year ranged between 1-26 (cats) and 1-209 (dogs). The data demonstrate a reduction in positivity over the years, although showing that rabies still circulates in this group, being extremely important the monitoring associated with the follow-up of rabies in bats and wild animals, since in the last years the variants diagnosed in dogs and cats were associated with these animals.

Keywords: domestic animals, urban rabies, environmental vigilance

EVALUATION OF THE INTERFERENCE OF CAT SERUM COMPLEMENT SYSTEM ON THE TITRATION OF RABIES VIRUS NEUTRALIZING ANTIBODIES BY THE RAPID FLUORESCENT FOCUS INHIBITION TEST (RFFIT) AND BY THE SIMPLIFIED FLUORESCENCE INHIBITION MICROTTEST (SFIMT)

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Some European countries require an International Animal Health Certificate and titers of rabies virus neutralizing antibody (VNA) > 0.50 IU/mL for the entry of pets (dogs, cats, and ferrets) into the destination country. From 2006 to 2011, at the Pasteur Institute of São Paulo/Brazil, a serological evaluation was carried out on more than 2000 serum samples of dogs and cats and the results have shown titers ten times higher than the minimum required in the cat sera samples. Heat-inactivation of cat sera was an important factor since the complement system can continue to act after inactivation at 56°C for 30 minutes. The aim of this study was to evaluate the usual method of complement system inactivation in cat sera samples and the interference in the evaluation of rabies VNA by the seroneutralization test in cell culture. For this, 60 serum samples of rabies vaccinated cats were submitted at different temperatures (56°C and 65°C) and different times (30 and 60 minutes), and the VNA titers were evaluated by the Simplified Fluorescence Inhibition Microtest (SFIMT) and also by the Rapid Fluorescent Focus Inhibition Test (RFFIT). The results were analyzed using the statistical unpaired T-test. The results demonstrated that a longer time of inactivation (60 minutes) does not show significant changes in serum neutralizing antibody titers in serum of felines; on the other hand significant differences of titers obtained with inactivation when increased at temperature (65 ° C) in both SFIMT (p-value 0.0002) and RFFIT (p-value 0.0007). It is also observed that samples that obtained a titration ≥ 0.5 IU/mL when inactivated with the routine protocol had a titration <0.5 IU/mL when inactivated with another protocol (3,34% SFIMT, 10% RFFIT). In conclusion, the serum inactivation at 65°C demonstrates efficient inactivation of the complement system showing a decrease in the VNA titers.

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Introduction

Rabies virus (RABV) belongs to Rabdoviridae family which includes three genera of animal viruses. By a monoclonal antibodies test against the viral nucleoprotein is possible to determine the antigenic variant. In Buenos Aires, the most commonly isolated variant is variant -4 (Tadarida brasiliensis). Though the major burden of samples are variant 4, there also exists other variant types related to other insectivorous bats (Myotis spp., Eptesicus spp., Histiotus montanus).

Myotis bat genus belongs to Vespertilionidae family and are worldwide distributed. It includes many species, in Argentina there were found at least 12 of this genus.

Materials and Methods

In December of 2017 we had a positive RABV diagnostic of a domestic cat that died with marked neurological symptoms. The Direct fluorescent antibody test with the brain tissue was negative though mouse intracerebral inoculation was positive. Considering the results of both assays, we send the sample for genetic tipification (RT-PCR) to the laboratory INEI ANLIS - "Dr. Carlos Malbrán". Sequences were performed by Sanger method using an automatic sequencer type AB13500. The alignment was made by "Clustal W", while phylogenetic analysis with MEGA 6.0, Neighbor joining and Kimura 2-P. The sequences were compared with the RABV antigenic variants of this region.

Results

The antigenic variant involved in this case was Myotis, being the first finding of this RABV variant in this laboratory. According to our database, Myotis bat genus is the 2% of the total bat samples received for Rabies diagnostic.

Discussion

Each rabies variant is maintained in a particular host, however, any variant can cause rabies in other species. Occasionally, a virus adapted to one species becomes established in another, and this is known as Spill-over hosts. Domestic animals' vaccination coverage is the most effective way to break the transmission cycle of the disease.

IMPORTANCE OF DOMESTIC DOGS (CANIS FAMILIARIS) IN THE EPIDEMIOLOGY OF ANGER IN THE STATE OF CEARÁ (2001-2016): A RETROSPECTIVE STUDY

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Introduction: With the implementation of rabies control actions in companion animals, through the intensification of rabies vaccination campaigns, it was possible to control the disease maintained and transmitted by populations of dogs in most of the country, however, there are still case records of the disease in these species in the state of Ceará. **Objective:** to carry out a situational diagnosis to know the importance of dogs in the epidemiology of rabies in the state of Ceará, from 2001 to 2016. **Methods:** The study comprised a descriptive study and data were collected from the State Health Department of Ceará, sectors of Epidemiology and Nucleus of Control of Vectors. **Results:** In the studied period, 16 cases of human rabies were reported, with the dog as the main transmitter in 68.75% of cases, however, there was a decrease in the participation of dogs over the years. 10,831 samples of dogs were sent for laboratory diagnosis of rabies, of which 293 were positive (2.70%); the percentage of positivity has decreased over the years. The cases of canine rabies were distributed in 56 municipalities and in all regions of the state, with greater concentration in the capital Fortaleza and Metropolitan Region. **Conclusion:** Canine vaccination coverage over the study period was higher than the goal recommended by the Ministry of Health, which is 80%. The number of human rabies due to dog aggression in Ceará has shown an increasing trend over the years. The findings show that rabies involving the canine species is present in the state of Ceará and represents a problem of importance for human, animal and environmental health, requiring strategies that are integrated and compatible with the social and regional context.

Keywords: Situational diagnosis, vaccination, canine rabies.

RABIES IN DOMESTIC FELINE WITH VARIANT OF *Desmodus rotundus*: AN ACTIVE SURVEILLANCE IN TABULEIRO DO NORTE / CE / BR

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Rabies is a serious mammalian transmitted viral encephalitis. In Brazil in 2017, 4 cases of feline rabies were recorded, all in the northeast region, 1 case (25%) from Ceará State in Tabuleiro do Norte municipality. In order to monitor the disease, in 2017 the municipality sent 63 samples for rabies examination. A positive diagnosis was made in 28.5% of the samples sent: bats (25.3%), felines (1.6%) and wild canids (1.6%). The rabies epizootic experienced by the municipality encouraged the actions of health surveillance and education about the prevention of the disease. In this sense, the present work aims to describe an unrestricted domestic feline rabies case with a viral variant of *Desmodus rotundus* in Tabuleiro do Norte in 2017. The episode happened in a municipality school. It was a male feline puppy, that struck a worker from the institution, who fed him. The case was reported and the patient treated. An epidemiological and laboratorial investigation was initiated, since the animal suspected of rabies died 10 days after the aggression. The feline, which had been discarded by the official, was unearthed, his marrow removed and sent for diagnosis, presenting a positive result. So, educational activity was carried out with students and school staff showing the risk of feeding unrestricted animals. In addition, vaccine blockade was performed on 125 dogs and 100 cats in the area. In this way, the circulation of wild rabies virus in Tabuleiro do Norte was detected thanks to the continuous work of surveillance, education and health mobilization with the population. It is worth emphasizing the importance of monitoring the spaces where bats are housed, in order to minimize the risk of dispersion and transmission of rabies. This work is fundamental for protection of the public health of the municipality, in view of the risk situation for zoonosis.

ATTITUDES TOWARDS DOG AND CAT BITES IN IN POOR NEIGHBORHOODS, BUENOS AIRES CITY

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Animal bites are of great importance for public health (psychological damage, scars, transmission of pathogens and even death). Approximately 8,000 bites are reported every year in Buenos Aires city (BAC). The knowledge of attitudes helps explain why among the possible practices for a subject in the face of a stimulus, that subject adopts one practice and not another.

The objective was to characterize the attitudes against dog and cat bites in poor neighborhoods of BAC.

An anonymous questionnaire was designed and 119 households were surveyed from the poor neighborhoods: Ramón Carrillo I-II and Villa N° 3 Nuestra Señora de Fátima (Villa Soldati, Commune 8, BAC).

Given the occurrence of a bite from a not own dog, the majority of the respondents would wash the wound (82.4%), disinfect it (64.7%) and request medical attention (85.7%); while given a bite of its own dog (84 dog owners), 86.9% would wash the wound with water and soap, 60.7% disinfect the wound and 70.2% request medical attention. Given the occurrence of a bite from a not own cat, the majority of the respondents would wash the wound (79.0%), disinfect it (63.9%) and request medical attention (77.3%); whereas given a bite of their own cat (30 cat owners), 80.0% would wash the wound, 66.7% disinfect it and 73.3% request medical attention. No statistical association was found between attitudes towards bites of dogs and cats (own/not own).

Dog and cat bites represent a constant challenge for public health. In this regard, a relaxation is noted at the community and health care levels shedding complexity to the prevention of associated pathologies such as rabies. In this way, the role of education for health promotion stands out as fundamental, as it is necessary to generate active, conscious, responsible, voluntary and permanent participation of the community.

PERCEPTIONS OF RISK OF DOG AND CAT BITES IN POOR NEIGHBORHOODS, BUENOS AIRES CITY

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Animal bites are of great importance for public health (psychological damage, scars, transmission of pathogens and even death). Approximately 8,000 bites are reported every year in Buenos Aires city (BAC). The perception of risk is an individual cognitive process, becoming evidence for the actions of the subject.

The aim was to characterize risk perceptions of dog and cat bites in poor neighborhoods of BAC. An anonymous questionnaire was designed and 119 households were surveyed from the poor neighborhoods: Ramón Carrillo I-II and Villa N° 3 Nuestra Señora de Fátima (Villa Soldati, Commune 8, BAC).

Of 119 respondents, 82.4% considered that a bleeding wound caused by the bite of a not own dog was a very serious event (value 4, on a scale of 1 to 4); 47.6% (84) dogs owners considered a bite caused by their own dog a very serious fact. On the other hand, of 119 respondents, 67.2% considered a bite by a not own cat as a very serious fact; while, of 30 households that owned cats, only 43.3% considered a bite by their own cat as a very serious fact.

Considering the perception of greater severity (value 4), a statistically significant difference (test for comparison of proportions) was found between a bite by a foreign dog with respect to a foreign cat ($p = 0.0112$), a bite by an own dog with respect to a foreign dog ($p = 0.0000$) and a bite by an own cat with respect to a not own cat ($p = 0.0177$).

The problem of dog and cat bites represents a constant challenge for public health. It is necessary to provide a qualitative sociological perspective for interpretation of the different perceptions involved in human-animal relationship, as well as the differences between the own/not own animals, in a specific sociocultural context.

RABIES IN PETS: VIRUS TRANSMITTED BY BATS

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Most of the cases received at the National Reference Laboratory are associated with hematophagous bats and bovines, equines and other production's animals are principally affected. A few cases of terrestrial rabies were also diagnosed in Argentina: V1 in Salta and V2 in Chaco and Formosa provinces during 2015 until present. The rest of the country has been considered terrestrial rabies free since the last case in the early 1980s. This led to neglect the mandatory annual vaccination of pets and consequently some cases of rabies in pets occurred associated with insectivorous bats because its potential consequent implications in public and animal health has not been taken into account.

Previous studies reveal that the rate of positive results of rabies in insectivorous bats received in the laboratory for analysis ranges from 3.1 to 5.4% and only 1% of natural bat population has been shown to be infected. Thus, the risk of contracting rabies from insectivorous bats is low. However, in the last years we have diagnosed many cases, specifically during 2018 due to a population awareness. This led to an increase in the number of bat samples processed in the laboratories belonging to the net of rabies diagnosis.

Rabies is diagnosed by direct immunofluorescence (DIF), isolation and the antigenic characterization is performed by indirect immunofluorescence (IFI) utilizing the reduced panel of eight monoclonal antibodies developed by the CDC. However, in some cases, antigenic analysis is unable to identify RABV isolates obtained from several insectivorous bat species because these isolates produce atypical reaction patterns and then the characterization is phylogenetic.

Four feline samples were received for characterization: AB 450 in November 2014 from La Matanza (Buenos Aires); AB 518 in August 2017 from San Miguel de Tucumán; AB 600 in January 2018 from Pinamar (Buenos Aires): these three samples resulted in V4 and the fourth sample, AB 537 received in November 2017 from Santa Fe, turned out an atypical reaction pattern, the phylogenetic characterization was *Myotis* sp.

PROFILE OF HUMAN RABIES POST-EXPOSURE PROPHYLAXIS IN RIO GRANDE DO SUL STATE, BRAZIL, BETWEEN 2012 AND 2016

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A descriptive study was carried out with secondary data from the database of the Brazilian Ministry of Health (SINAN). Data from 163,978 records of human rabies post-exposure prophylaxis (PEP) from Rio Grande do Sul State (RS), from 2012 to 2016, were analyzed. The profile of potential human exposure to rabies in RS showed that around 63% of the cases were registered between the ages of 15 to 65 years old. The most frequent type of potential exposure to rabies virus was animal bite, with 82.5% frequency, particularly as a single wound (61%). The aggression was more frequent in hands and feet (32%) and lower limbs (37%). The predominant aggressor species was canine (87.7%), followed by felines (8%), domestic herbivores (2%), chiroptera (0.45%), primates (0.16%) and foxes (0.05%). In 73% of the cases, aggressive animals were described as healthy, and the dogs and cats that could be observed represented 74%. Concerning the treatments, the "animal observation" (34.4%) and the "observation + vaccine" (38.7%) were the most indicated for the patients. In RS, 82% of the patients sought medical care one day after the animal aggression, which may show a greater perception of the risk of rabies exposure. Treatment involving rabies immunoglobulin and/or vaccination was performed in 50% of the aggressions by dogs and cats that were mostly observable and healthy. The identification of the profile of the PEP patients can direct actions of health education. Considering that the incidence rate of rabies in dogs and cats is very low in RS state, there would be a great saving of immunobiologicals if the observation criterion of dogs and cats was better used.

EPIDEMIOLOGICAL SITUATION OF RABIES IN MARANHÃO, BRAZIL, IN THE PERIOD FROM 2015 TO 2017

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Rabies is an anthroponosis transmitted to humans by the inoculation of rabies virus contained in the saliva of infected mammals, mainly by means of biting. It is an acute viral encephalitis that presents two main cycles of transmission: urban and wild. In Brazil, although there are several effective prevention measures, rabies continues to occur persistently and the state of Maranhão, in 2017, got into a state of alert. The objective of this study is to analyze cases of rabies in humans, dogs, cats, hematophagous bats, non-hematophagous bats, wild canids, cattle, equine, non-human primates, in the period of 2015 to 2017, in Maranhão. During this period, 25 cases of rabies were registered in Maranhão. The highest number of cases was verified for bovine rabies (48%) in São Domingos, six cases in 2015 and six cases in 2016. Then, the highest number of cases was recorded for canine rabies (32%), one in São Vicente Ferrer, one in Humberto de Campo and one in Viana in 2015, one in Central do Maranhão in 2016 and two in 2017 in São Vicente Ferrer and two in Santa Inês. Feline rabies was registered in the municipality of Viana (8%) in 2015. And in 2017, one case of equine rabies (São Domingos) and two cases of rabies in hematophagous bats (*Desmodus rotundus*) (Raposa) were recorded. These data show that most of these cases of rabies are concentrated in the baixada maranhense, and actions and guidelines are necessary, especially in relation to the prophylaxis and indications for the control of this disease in dogs, cattle and hematophagous bats. This information is important in the timely organization of mitigation actions against cases of rabies in animals, seeking the prevention of human cases, to prevent the spread of the virus among the different species.

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Rabies is an important worldwide distribution zoonosis, caused by a virus of the rabdoviridae family, genus Lyssavirus. It presents variable symptomatology that differs between species of mammals, being differential diagnosis of neurological diseases. For laboratory diagnosis, samples are collected from the central nervous system of the animal. The method used is Direct Immunofluorescence (DFI) recommended by the World Health Organization (WHO), because it is fast, sensitive and specific, in which it is based on the antigen - antibody reaction. The objective of this study is to know the prevalence of rabies in animals in the period from 2013 to 2016, through laboratory diagnosis using the DFI method. In the period from 2013 to 2016, a total of 1,824 samples were received at the Animal Rage Diagnostic Laboratory, of which 163 were positive. The positivity is distributed in the following species: fox, sagui, bat, dog, cat, bovine, raccoon, asinine, Equine and goat / sheep. The overall prevalence was lower in 2014, 4.13%, and the highest was observed in the year 2016 with 13.38%. The laboratory diagnosis of rabies is of great value for the confirmatory diagnosis in animals, being support to the municipalities of the state. The prevalence of animal rabies has increased over the years with the perception in wild species and animals of production. We observed a change in the epidemiological profile of the disease, with a reduction in the number of positive dogs and cats resulting from the annual rabies immunization campaigns. It is worth noting the progress of positivity in wild animals, especially the population of bats present in urban areas. There is a need for a gradual increase of municipalities that send samples for diagnosis.

MUNICIPALIZACIÓN DEL PROGRAMA DE PREVENCIÓN Y CONTROL DE LA RABIA, UNA OPORTUNIDAD PARA MANTENER LA AUSENCIA DE LA ENFERMEDAD

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La rabia es una zoonosis de importancia para la salud pública ya que se relaciona con la convivencia diaria que se tiene con los animales de compañía, el panorama epidemiológico destaca la reducción de los casos de rabia canina y humana transmitida por el perro debido a la prevención que se lleva a cabo mediante actividades de promoción y educación para la salud. Promover la coordinación con los municipios, es vital para disminuir el riesgo de que se presenten casos en seres humanos. Los municipios deben contribuir con centros de atención canina o en su caso realizar actividades orientadas a resolver los problemas que provocan los perros y en menor proporción los gatos; además de realizar otras actividades como son la captura de perros callejeros, recolección y observación de animales agresores, la eliminación de los no reclamados, obtención de muestras y servicio de diagnóstico de animales sospechosos, en algunos casos representan el primer contacto con las personas agredidas. Sin embargo, es necesario prestar atención a otras actividades para enfrentar este problema, como es la vacunación antirrábica y el servicio de cirugías para la esterilización de mascotas. Por lo anterior es determinante que en base a las leyes federales y reglamentos estatales los municipios adopten sus propias normatividades para contribuir en aplicar acciones que ayuden a mantener la ausencia de casos tanto en animales como en seres humanos.

EVOLUCIÓN CRONOLÓGICA DE ESTRATEGIAS, AVANCES Y RESULTADOS EN EL CONTROL DE LA RABIA HUMANA TRANSMITIDA POR PERRO EN LA JURISDICCIÓN SANITARIA 7, DE IZÚCAR DE MATAMOROS, PUEBLA

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En México durante 1709 se presentó la primera epizootia de rabia en perros, registrando casos año tras año tanto en humanos como en animales. Por lo anterior inicia el diseño de estrategias para el control de la enfermedad sin una metodología establecida. El estado de Puebla por más de cinco décadas convivió con la rabia y lo identificó como un problema de salud pública. La incidencia de rabia canina llegó a registrar en los años noventa hasta 350 casos y superar 10 defunciones en un año. A efecto de mejorar el panorama epidemiológico en 1968 se lleva a cabo la primera campaña de vacunación antirrábica masiva, la cual se extiende en toda la nación a partir de 1990. Reduciendo paulatinamente los casos de rabia en México. El estado de Puebla en el año 2000, presentó la última defunción por rabia, como resultado de la elaboración de estrategias para el control de la enfermedad en perros, por ser el principal transmisor de la rabia a humanos, y entre otras, la elaboración de un plan anual de trabajo en concordancia con el plan nacional de salud y actualmente con el proceso optimo como estrategia de evolución, con la intención de cuidar los logros. En Izúcar de Matamoros, la aplicación de cada una de estas estrategias en las áreas de riesgo fue paulatina pero contundente y no se dejaba la zona hasta haber alcanzado el propósito de controlar la rabia canina. El objetivo es desarrollar estrategias inherentes a la vigilancia epidemiológica, promoviendo la intervención de instituciones y municipios, favoreciendo la concientización a la población con una mejora en la atención integral de los eventos que se presenten, para sostener la ausencia de rabia entre las personas, perros y gatos.

CONCURSO DE DIBUJO INFANTIL “CONTROL DE LA POBLACIÓN DE MURCIÉLAGO HEMATÓFAGO”

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Introducción. El Comité Estatal de Fomento y Protección Pecuaria del Estado de Hidalgo A.C., opera la campaña de Rabia en Bovinos y Especies Ganaderas. Como parte de la difusión, se han proyectado concursos de dibujo dirigidos a la población infantil.

En 2015 se realizó el concurso estatal “Día Mundial Contra la Rabia” dirigido a alumnos de educación primaria con el tema “La rabia en el ganado” con la participación de 58 escuelas en 30 municipios, se recibieron 1,070 dibujos, premiando a los 6 mejores.

Objetivo. Fomentar en la población infantil el conocimiento y la importancia de la rabia en bovinos y especies ganaderas y las afecciones en la salud, así como el conocimiento del murciélago hematófago como transmisor de este padecimiento.

Problemática. El 80% de los casos de rabia en el estado, se presenta en ganado sin un calendario de vacunación adecuado o nunca vacunados, pertenecen a pequeños productores o dueños que los mantienen en traspatio, sin proporcionar la atención apropiada debido a la falta de conocimiento de las medidas de prevención y control que se pueden aplicar para el control de la rabia.

Métodos. Se proyecta la realización de un concurso de dibujo infantil en septiembre del 2018, dirigido a niñas y niños de primero a sexto grado en las escuelas primarias del estado.

Resultados. Se busca que esta estrategia de difusión, permita que la población infantil comparta con los adultos en sus casas las medidas de prevención y control de la rabia transmitida por murciélagos vampiros que se respeten a otros murciélagos no vampiros y benéficos y que ello colabore a fortalecer los conocimientos sobre rabia de los pequeños productores pecuarios en el estado de Hidalgo.

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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 lack of attendance 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 central point vaccination clinics were recorded. The Kaplan-Meier test was applied to determine relationships between survey variables. Cox regression model was used to examine factors influencing participants' WTP and WTW. A total of 748 households from 8 communities were enrolled. Respondents were predominantly female (54.4%), with a median age of 45 years. The number of owned dogs reported was 926, yielding a human dog ratio of 5.2:1. The majority of dogs (87.2%) were acquired for security purpose and 49% were allowed to roam freely. 42.0% of dog owners reported that they were unable to walk their dogs on a leash. To achieve 70% vaccination coverage, people were willing to pay 15.9 gourdes (0.25 USD) and walk 75 meters to vaccinate their dogs. We also found that WTP and WTW was influenced by economic, demographic, and dog-ownership status. Higher income, affection for one's dog, and owning a free-roaming dog were all associated with a higher willingness to pay and walk for vaccination. In conclusion, the lack of familiarity with leashes and propensity for dogs to roam freely are barriers to successful fixed point vaccination methods in Haiti and alternative methods such as door-to-door, capture-vaccinate-release or oral vaccination should be explored. There may be some prospect for fee-for-service vaccination in Haiti, however if implemented it should be optional as to not discourage dog vaccination.

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Rabies Expert On-Demand (REOD) is a service provided by Centers for Disease Control and Prevention (CDC) to assist public health practitioners, clinicians, and the public (domestic and international) to interpret rabies prevention guidelines. Inquiries are received through phone and email. REOD is staffed by the CDC Poxvirus and Rabies Branch (PRB) and includes epidemiologists, laboratorians, veterinarians, and physicians. Recommendations provided by REOD are derived from the Advisory Committee on Immunization Practices and the National Association of State Public Health Veterinarians compendiums for rabies prevention. Due to the diverse and often complex nature of rabies exposures, prescriptive guidance is not always found in these documents; REOD provides the experience and expertise to assess each unique situation and provide evidence-based guidance to stakeholders. An inquiry tracking form and Microsoft Access database were developed to document all inquiries. Records of REOD inquiries were summarized for August 2017- 2018 to determine the frequency of inquiries by month, category and location. Over a 12-month period, REOD received 1,022 inquiries, 434 by phone and 588 by email, 866 were domestic and 156 international. Peak inquiries (n=124) occurred during July. The most frequent inquiry pertained to risk assessment and management of rabies exposures (n = 423), followed by consultation for deviations in the recommended post-exposure prophylaxis regimen (n = 106), requests for testing assistance (n = 106), and consultation for suspected human rabies (n = 68). An additional 80 inquiries were received regarding exposures and post-bite treatment occurring internationally. CDC's PRB REOD is a global resource for consultation related to managing rabies exposures, diagnostic issues, and rabies control strategies. REOD is a heavily utilized CDC service, as the demand for up-to-date rabies guidance remains high. REOD fulfills a critical role for the interpretation and consultation on rabies prevention guidelines for state agencies, international agencies, and the public.

ANTIGENIC AND MOLECULAR TYPING OF RABIES VIRUS STRAINS ISOLATED AT THE INSTITUTO DE ZONOSIS LUIS PASTEUR (IZLP) FROM BUENOS AIRES, IN THE PERIOD 2007- 2018

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Since created in 1886, the Instituto de Zoonosis Luis Pasteur (Buenos Aires) has pioneered both the production of antirabic vaccines as well as rabies diagnosis. Since 1992 a monoclonal antibody panel against the viral nucleoprotein was introduced to typify circulating variants. In 2008 a Reverse Transcription – Polymerase Chain Reaction (RT-PCR) technique was employed in parallel for diagnosis in decomposed samples. In 2010 this technique was coupled with Sanger sequencing for molecular typing. Positive samples submitted to the IZLP between 2007-2018, were typed and strains were associated to their geographical distribution.

Antigenic characterization was performed by Indirect Immunofluorescence using a 19 monoclonal antibodies panel (CDC, USA). Viral RNA extraction was carried out using column-based commercial kits. RT-PCR was performed using the OneStep Kit RT-PCR (Qiagen, Germany) obtaining a product of 259 bp. Sequencing reactions were carried out using BigDye Terminator v3.1 (ThermoFisher, USA), and reads were obtained using the Applied Biosystems 3500 Series Genetic Analyzer (ThermoFisher, USA).

In the studied period, 328 (7.5%) out of 4348 diagnostic submissions were positive, 284 (86%) of which were typed. The most prevalent variant V4 (59.71%) linked to the bat species *Tadarida brasiliensis* but found also in four dogs, three cats, and a red fox. The subsequent most frequent variants were V3 and V3a, associated to the vampire bat *Desmodus rotundus* (23.31%), followed by V1 (8.48%), *Myotis* spp (2.82%) with one case in a dog, *Eptesicus* spp (1.41%) with 2 cases in cats, *Histiotus* spp (1.41%), V6 or *Lasiurus* spp (1.76%) and V2 (1%). This study depicts rabies virus distribution in reservoirs from Argentina since 2007 to date. A total of 11 spillover events linked to insectivorous bats and a human case with V1, remark the importance of holding antirabic vaccination programmes in canines and felines.

ANALYSIS OF HUMAN ANTIRRADIC PROPHYLATIC TREATMENT AT 15 CRES - CRATEÚS, YEARS FROM 2013 TO 2016

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Rabies is an acute disease viral encephalitis, mammalian transmitted. Transmission occurs when the virus contained in the saliva and secretions of the infected animal penetrates the tissue, mainly by biting and, more rarely, by scratching and licking mucous membranes and / or injured skin. The objective of this study is to analyze the treatment of human rabies in the population of 15 CRES - Crateús municipalities, from 2013 to 2016. The study has a quantitative, non - experimental character. The research was realized in the 15th Regional Health of the State of Ceará, Brazil, composed of 11 municipalities: Ararendá, Crateús, Independência, Ipaporanga, Ipueiras, Monsenhor Tabosa, Nova Russas, Novo Oriente, Quiterianópolis, Poranga and Tamboril. During the study period, 2.933 people were assaulted, of which 2.842 were treated, or 96,89%. 5.775 doses of rabies vaccine were used in treated subjects. Of the aggressions, 1.956 were caused by dogs, or 66,68%. 847 were caused by cats, or 28,87% .134 were caused by other animals, such as monkeys, cattle, pigs or rodents, a total of 4,56%. The number of aggressions by dogs and cats was 95,44%, and of these animals, 53,12% were observed. It is possible to observe a large number of these animals. According to the human prophylactic treatment scheme, under the guidance of the Ministry of Health of Brazil, people with superficial aggressions, whose animals (dogs and cats) can be observed, do not need to undergo prophylactic treatment. We suggest that municipalities, in partnership with the State, conduct continuous training in their health professionals.

PERFIL EPIDEMIOLÓGICO DE LAS DENUNCIAS POR MORDEDURA OCURRIDAS EN EL MUNICIPIO DE ITUZAINGO, EN EL PERÍODO ENERO-DICIEMBRE DE 2017

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Introduction: Animal bites pose a public health problem for children and adults around the world as they are a major cause of morbidity and mortality. According to studies, dog bite lethality rates are higher in low and middle income countries. Rabies is a problem in many of these countries, and post-exposure treatment may not be available and there may not be adequate access to health care.

Method: A descriptive study of the complaints of dog bites received at the zoonosis center of the Municipality of Ituzaingo was carried out from 01/01/2017 to 12/31/2017.

Results: 500 complaints were registered during the study period. 56% were female. 25% corresponded to children under 14 years of age. The neighborhoods with the most complaints were Villa Leon and Villa Zona Norte. 80% of the bites occurred in the head corresponded to children under 10 years. In 33% of the accidents street dogs were involved. Almost 60% had no record of rabies vaccine. In 62% of the complaints, the corresponding anti-rabies observations could be made.

Conclusions: It was observed that the number of children under 14 years of age bitten is high as well as the possibility that bites occur in regions of the head. It is known that there is a subnotification in part due to the circuit established to make the complaints.

Based on these data, a new notification circuit is proposed with the aim of being able to work in a network with other anti-rabies centers

RETROSPECTIVE STUDY OF HUMAN RABIES CASES CONFIRMED BY LABORATORY DIAGNOSIS AT THE INSTITUTO PASTEUR, SÃO PAULO, BRAZIL, IN THE PERIOD 2003-2009

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Over the years, a decrease in the number of cases of human rabies transmitted by dogs and a considerable increase in transmission by wild animals was observed. The aim of this study was to evaluate available data of human rabies cases diagnosed at the laboratory of Instituto Pasteur, in the period 2003-2009. We assessed the diagnosis request files and the data registered in the computerized system. Human rabies cases were considered positive when confirmed in at least one laboratory technique. The data were analyzed descriptively considering the epidemiological variables as transmissions as characteristics of the person attacked, the aggression, the aggressor animal, the geographic location and the seasonality. In the evaluated period, 31 cases were detected positive for human rabies, of these 61% are men, 25.9% are children from 0 to 10 years, 83.9% suffered aggression in the Northeast region, and the Maranhão state presented the highest occurrence, with 71%. In the rural area more human rabies cases (38.8%) occurred than in the urban area (12.9%). The most frequent form of aggression was the bite with 48% and the most commonly reached place were the feet, presenting in most cases single and deep wounds (16.2%). In this period, the bat had greater importance in the transmission of human rabies, responsible for 33.2% of the cases. Spring was the season with the highest number of cases (38.7%). It is important to note that among the analyzed datasets, a high frequency of uninformed data was observed, ranging from 29 to 45.2%. The number of human rabies cases diagnosed declined in the considered period. On the other hand, a greater number of human rabies transmitted by bats was observed, suggesting a tendency of change in the epidemiological profile in relation to the animal species involved in the transmission.

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In 2017, the two CFIA rabies laboratories tested 2775 samples for rabies. The majority of samples came from animals that potentially exposed a person (66.8%); all other samples had only domestic animal contact (19.7%) or no documented contact (13.5%). Two hundred and thirty-nine samples (8.6%) tested positive. Of these, 132 (55%) were confirmatory tests on wildlife surveillance samples, which were initially analysed in provincial laboratories by either the direct, rapid, immunohistochemical test for rabies or immunohistochemistry (IHC). An additional 6 cases 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=2768); 3 were tested by IHC, and 4 by quantitative RT-PCR (human suspect cases, all negative). Raccoon-variant rabies outbreaks, originally detected in 2014 and 2015 in New Brunswick and Ontario, respectively, continued in 2017. With similar levels of surveillance between years, New Brunswick cases remained constant (3 in 2016, 4 in 2017), whereas case numbers significantly decreased in Ontario (258 in 2016, 119 in 2017). This latter observation likely explains the significant decrease in the proportion of positive samples from 2016, when 12.8% tested positive. However, given these outbreaks, not surprisingly raccoons again accounted for the highest proportion of cases in 2017 (36%), followed by skunks (26%), bats (23%), and foxes (6.7%). Among domestic animals, rabies was detected in 7 dogs, 7 bovines, and 4 cats, the result of spill-over of skunk-variant virus in western Canada (n=7), either fox-variant virus (4 bovines) or raccoon-variant virus (1 cat) in southwestern Ontario, and fox-variant virus in northern regions (5 dogs). No spill-over cases due to bat variant viruses were detected in terrestrial species in 2017. Although the majority of cases were detected in wildlife species (222/239, 92.9%), domestic species accounted for 44.5% (1235/2775) of specimens submitted for testing.

EPIDEMIOLOGICAL SURVEILLANCE OF RABIES IN INSECTIVOROUS BATS FROM NORTH PATAGONIA, ARGENTINA

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Introduction: In the Patagonian region of Argentina there have been no human cases of rabies. While there was an imported case of rabies in a dog, there were no other cases in domestic animals. However, positives for rabies were reported in some wild animals such as foxes (terrestrial cycle) and bats (aerial cycle).

In the easternmost portion of Río Negro (North Patagonia, Argentina), bats are responsible for completing the aerial cycle of rabies virus: *Tadarida brasiliensis* is the most common species in this region. These bats have the particularity of having adapted very well to urban environments, living gregariously in colonies of thousands of individual and having a predilection for human dwellings.

Materials and methods: The passive surveillance system is based on the identification and analysis of bats captured by personnel of the Regional Epidemiology and Environmental Health Unit of the Ministry of Health of the Province of Río Negro. Sampling design was conducted from December 2008 to August 2018. Data were also collected on the contact with other animals and people.

Results: Two different techniques have confirmed 15 positive cases of rabies from a total of 146 samples obtained in this study. With the exception of three bats belonging to the genus *Myotis* ssp., all remaining individuals were classified as *Tadarida brasiliensis*. In all cases they were found and captured in urban areas, inside the houses or in peridomestic sites, generally during the day. The identification of positive cases was performed at the Luis Pasteur Institute of Zoonoses of the Autonomous City of Buenos Aires.

Conclusions: The recognition of insectivorous bats as reservoirs of the disease is of great importance due to the close contact with humans when they living in their dwellings, allowing us to take preventive measures towards the population.

Keywords: epidemiology, rabies disease, insectivorous bat

SPATIAL NON-RANDOMNESS OF SKUNK AND SKUNK RABIES DISTRIBUTION IN THE CENTRAL GREAT PLAINS

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The persistence of rabies among Striped Skunks (*Mephitis mephitis*) poses a continuing public health threat in North America thanks to spillover potential of the virus from skunks to a wide range of other hosts. Understanding the geographic distribution of the skunks and the pathogens that they host is a crucial first-step to understanding the ecology of skunk rabies, and eventually for safeguarding public health from this disease. Although environmental factors (e.g., climate) are well-known as constraints on the distributions of vertebrates, whether or to what degree environmental parameters limit the distribution of rabies virus to a subset of skunk distributions remains an unanswered question. In this study, we tested whether rabies-infected skunks were distributed randomly with respect to environmental parameters across the geographic distribution of skunk populations in the central Great Plains region (Kansas and Nebraska), based on diagnostic records received at the Rabies Laboratory at Kansas State University for testing between 2012 and 2017. Ecological niche models for skunks were constructed based on different metrics of temperature, precipitation, and relative humidity, drawn from the remotely-sensed PRISM data archive at 4 km spatial resolution. Importantly, rabies-positive skunks were found under biased environmental conditions with respect to 6 of 9 climatic dimensions that we explored. Indeed, whereas areas most suitable for skunks were in the southeastern part of the study area, the most suitable areas for rabies-positive records were in the northwestern sectors. This study represents the first of several steps toward understanding the spatiotemporal dynamics of skunk rabies in the central Great Plains of the US.

EVIDENCE OF THE AERO-TERRESTRIAL LINK IN RABIES VIRUS ISOLATIONS FROM ARGENTINA

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Canine rabies control has been achieved in Argentina during the 80s. Despite this, positive cases are sporadically detected in border areas. In addition, some positive cases in cats and dogs with no evident epidemiological link were registered. During the 90s, with the incorporation of Monoclonal Antibodies from the CDC (USA) to the Instituto de Zoonosis Luis Pasteur, allowed the identification of bat-borne rabies virus in terrestrial animals. The first of such cases was registered in 1992, a virus variant 3 present in a Chacoan crab-eating fox (*Cerdocyon thous*), a natural vampyre bat predator. Since then, with the incorporation of sequence-based typing, a total of 14 spillover events were detected. Most isolations were remitted by laboratories from the Red Nacional de Rabia. The majority of cases were from the province of Córdoba (42.8 %) and Buenos Aires (28.5 %), the remaining (28.4 %) involves CABA, Chaco, Salta, Río Negro in similar proportions. Variant 4 (71% of total isolations), was detected in 75% of dogs and cats cases, and in an Andean fox (*Lycalopex culpaeus*). Virus variants associated to *Eptesicus* spp (14.2 % of total isolations) were found in 33.3 % of cat cases, while those associated with *Myotis* spp (7.1 % of total isolations) were found in 16.6 % of dog cases. Most cases occurred during temperate/warm months in the intervals 1992-2002 (21.4%), 2008-13 (50.0%) and 2017-12 (28.6 %). Spillovers are not frequent but are still possible events. Together with the recurrence of urban canine rabies in Salta and Jujuy (the two provinces limiting with Bolivia), spillovers enhance the need for maintaining epidemiological surveillance, vaccination coverage in cats and dogs and the diffusion of the problem to both the population and health professionals.

SURVEILLANCE OF POTENTIALLY RABIES ACCIDENTS IN BUENOS AIRES, ARGENTINA

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INTRODUCTION. Rabies is a neglected disease with a great impact on public health. The World Health Organization (WHO), together with the World Organization for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO) and the World Alliance for the Control of Rabies (GARC) have united with the common goal of eliminating human rabies and controlling this disease in animals. In the province of Buenos Aires, Argentina, the potentially rabies accidents (PRA), in people, by domestic animal bites represent a serious sanitary problem, due to the possibility of transmission of variant rabies virus 1, coming from canines or felines that migrate of endemic areas or because of the risk of the variant passage of insectivorous bats due to the spillover effect. Therefore, the monitoring and characterization of the PRA are essential to develop adequate prevention strategies.

MATERIALS AND METHODS. A survey of the PRA, notified to the Urban Zoonoses Department of the Ministry of Health of the province of Buenos Aires, between 2015 and 2017, was carried out and classified according to age range, sex, domicile and whether they received post-exposure anti-rabies treatment.

RESULTS AND DISCUSSION. During this period, 117,666 PRA were recorded. 90% came from municipalities of the Buenos Aires Conurbation, where 73% of the human population of the province of Buenos Aires is centralized. Of these cases, 58,739 people (49.92%) received post-exposure anti-rabies treatment, according to current health guidelines, according to the anatomical location, severity of the injury, and characteristic of the aggressor animal. It was also observed that 53.78% were older than 21 years old, 18.92% were children from 6 to 14 years old, 14.01% children from 1 to 5 years old and 13.29% from 15 to 20 years old, without significant difference between both sexes.

PHYLOGENETIC ANALYSIS OF WHOLE GENOME SEQUENCE IMPROVES THE CHARACTERIZATION FOR RABIES VIRUS IN CATTLE

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Bovine rabies is a big economical problem in Latin America, as we know main transmitter is the vampire bat (*Desmodus rotundus*). This study characterized four rabies virus coming from cattle using whole genome sequences (WGS) from Queretaro, Mexico. Samples were diagnosed by fluorescent antibodies as positives and antigenic characterization was AgV11 genotype 1, provided by the Animal Pathology Laboratory in Queretaro, Mexico. The WGS of the rabies virus was obtained using the Illumina NextSeq 500 platform and reconstructed with genome BR-DR1 (GenBank number AB519642.1) as reference. A BLAST analysis was used to identify sequences in the GenBank with 90% similarity with the four Mexican strains, which yielded 22 analogous. Afterwards, the sequences were aligned using the method Clustal W v1.83 (Multiple Sequence Alignment analysis). The phylogenetic analysis was performed with MEGA 6.0. with the Neighbor-Joining method and bootstrapped to 1000 replicates. The phylogenetic tree showed that three large and seven small clusters were formed using 26 GenBank and Mexico complete sequences. The largest cluster grouped strains from different species in South America, such as Brazil and French Guyana. The second cluster grouped the five complete sequences from Mexico, suggesting common source of infection. The WGS analysis shows that the circulating virus in this geographical region is genetically stable and that it is transmitted by *Desmodus rotundus*. Additionally, the phylogenetical tree generated with WGS showed 22 out of the 23 internal nodes had bootstrap values higher than 90%, whereas partial sequences of N, P and G genes had a phylogenetic resolution in the range of only 2-13 bootstrap values higher than 90%. The use of the WGS improves the phylogenetic inference to understand the spread of rabies virus in cattle and, this gives a valuable and more complete information that can be generated for improving a deepest molecular epidemiology in Mexico.

POSTER PRESENTATIONS

TUESDAY 30 OCTOBER 15:00 - 16:30

Picotto L., Vita C., Cavalitto S., Pecoraro M., Sguazza G.

Laboratorio de Virología (FCV-UNLP) y CINDEFI (CONICET-UNLP)

Presenter: Leandro Daniel Picotto

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Rabies is a serious disease which causes a fatal form of encephalomyelitis in humans and animals when treatment is not administered. The incidence of human rabies can be reduced controlling canine rabies, through vaccination programs and sacrificing stray dogs. In Argentina, vaccination campaigns use vaccines produced in the brain of suckling mice. Production of this vaccine involves handling live virus and is expensive. In addition, it has been discouraged by the World Health Organization. Rabies is a worldwide disease which mainly affects developing countries. Thus, an efficient and affordable solution is needed. In this context, the production of the most immunogenic proteins of rabies virus (the glycoprotein and the nucleoprotein) was studied in bacteria and yeasts, as an alternative to the current vaccine production method. These expression systems were selected due to the technical advantages they have: easy handling, high levels of protein expression and simplicity to scale. Results show that the recombinant rabies proteins were expressed and purified easily, and can be used as a safe source of antigen in the production of a subunit vaccine, for the prevention of the disease. The immunogenicity of the recombinant proteins was assessed by potency assay of the National Institutes of Health, but protection was not achieved in mice. However, the recombinant proteins were able to produce specific antibodies against the native proteins of the virus, although in a lower titer than necessary to generate protection against a viral challenge. These results are encouraging for the future, because through new studies, by increasing antigen concentrations or with the use of other adjuvants, it would be possible to reach specific titers of antibodies against rabies capable of producing protection against a viral challenge.

EPIDEMIOLOGICAL CONSIDERATIONS OF RABIES IN CIUDAD AUTÓNOMA DE BUENOS AIRES

Faro, Emilio; Blanco, Carlos; Cuatrin, Jorge

Ministerio de Salud de la Nacion. Argentina - Universidad de Buenos Aires. Facultad de Ciencias Veterinarias. Argentina

Presenter: Emilio Faro

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In Argentina anti-rabies vaccination is mandatory in canines and felines and possible susceptible species throughout the territory (Law 22953/83). The population of canines and felines at Ciudad Autónoma de Buenos Aires in 2013 was estimated at 440,000 animals inhabiting a total of 357,000 homes.

An epidemiological study of the vaccination status against rabies in the population of dogs and cats attended by private veterinarians of the CABA was made, based on a sample taken from 4900 clinical histories. The study was during 2013 Sampling was planned in a cluster scheme selecting between 3 - 5 veterinarians in each of the city districts.

Approximately 50% of the animals attended at veterinarians are vaccinated (or figure registered as vaccinated), this percentage being 52% in dogs and 45% in cats, these differences being significant. ($p < 0.05$)

We observed that there is a statistical association between the castration of animals and their vaccination, with a proportion of 55% of vaccinated animals in castrated animals compared to 47% in non-neutered animals. ($p < 0.05$ in Pearson's χ^2 test).

The estimated proportion of vaccinated animals showed a heterogeneous distribution in different city districts.

M. Shulpin, A Chupin, P. Evdokimof, A. Metlin
FGBI ARRIAH

Presenter: Mikhail Shulpin
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The Russian Federation is permanently affected by rabies. The most difficult epizootic situation is present in the Central and Volga parts of Russia. In the Eastern part, several administrative units such as Irkutsk, Magadan, the Sakha, Primorsk, are considered rabies free territories. Despite that fact, there is a risk of rabies introduction from neighboring territories. In 2017, rabies was notified in the Buryatia, where rabies was not notified since 2012. To determine the possible ways of introduction of rabies virus, a genetic analysis of the rabies virus isolates was carried out.

The nucleotide sequence of the N gene rabies virus was length of 1066 nucleotides determined for 7 isolates. The sequence of "neighboring" isolates published in GenBank were used for phylogenetic analysis, as well as the sequence of isolates identified earlier in the Buryatia.

As follows from the phylogenetic analysis, the new isolates belong to two groups with geographical identity. Differences between the groups of isolates were 0.4%. Maximum homology to the new isolates were shown for isolates of 2015 y from the neighboring Zabaikalsk. Differences were 0.5% (5 nucleotide substitutions), while all other isolates differed for more than 0.7%.

It should be noted that the new isolates from 2017-2018 differ from the isolates detected in the outbreak of rabies in Buryatia in 2011-2012 by more than 1.3%. Therefore, despite the identical geographical location, it can be suggested that the outbreak of 2017 is not a continuation of the outbreak of 2011-2012, and has an origin from another source.

Thus, the isolates of the rabies virus from 2017-2018, identified in the Buryatia, presumably came from territory of the Zabaikalsk. The presence of at least 2 groups of isolates indicates the gradual evolution of the virus and suggests that the virus was introduced to these places a few years.

DESCRIPTIONS OF LABORATORY DIAGNOSIS OF BOVINE RABIES IN RIO GRANDE DO SUL STATE, BRAZIL, FROM 2011 TO 2017

Julio César Almeida da Rosa, José Carlos Ferreira, Laura Lopes de Almeida, Carla Rosane Rodenbusch, Alexandre Cenci, Bruno Egidio Cappilari, Giovana Dantas.

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Presenter: Julio Rosa

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Rabies remains one of the most important worldwide zoonosis in Brazil. In the Rio Grande do Sul (RS) state, Brazil, the disease is one of the main causes of death in bovine herds. The confirmation of suspected rabies cases upon clinical examination depends on specific laboratory diagnosis. The Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF) is the reference laboratory in RS for rabies diagnosis. The aim of the present study was to present a description of rabies diagnostic in cattle, in the last 7 years, in RS. Immunofluorescence direct and biological assays for confirming rabies cases were used. In the study period, 1178 samples of brains of cattle presenting neurological symptoms compatible with rabies, of which 655 (56%) were confirmed in the laboratory. The frequency of positive cases on a yearly basis varied between 35 and 65%. Samples were from 191 municipalities, of which 123 (64%) had at least one confirmed case and 68 did not have any such case of rabies. The five municipalities that sent most of the samples for analysis were also the ones that had the most positive results: Viamão (37/76), Gravataí (31/43), Montenegro (29/46), Camaquã (25/40), and São Lourenço do Sul (24/43). A large number of the municipalities presenting rabies cases in cattle were in the metropolitan region of Porto Alegre and in southeast RS, which are areas of high and intermediate risk for the disease, according to a previous epidemiological modeling study. The large number of rabies in cattle in RS underscores the importance of the disease and the high risk of exposure, both to humans and animals. The results presented here may help define control and prevention actions against rabies in RS.

RABIES SURVEILLANCE IN CORDOBA ARGENTINA

Buteler, Florencia; Gonzalez, Patricia; Jose, Gallegos; Lopez, Laura

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Presenter: Florencia Buteler

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The present study reports the effect of surveillance system strengthening in the prevention and control of rabies in Córdoba (2017-2018), Argentina.

In 2017, we tested 127 samples, 15 (11.8%) were positive for rabies virus (27.8% animals of economic importance (AIE), 8.3% felines, 13.3% bats and 3% dogs). In 2018, 201 samples were analyzed, 30 (14.9%) were positive (37.5% AIE, 16.4% bats and 4.8% dogs). All cases were confirmed at least by two different tests. The increase of positive cases in AIE, in the northwest of Córdoba, represents a problem of economic importance in this region. Variant 3 was identified in all AIE and hematophagous bats and variant 4 in non-hematophagous bats, dogs and cats.

Otherwise, we registered 192 bitten people in 2018, 41% were derived for post-exposure prophylaxis (PEP), while in 44% of the cases the animals antirabic observation could be performed. The remaining 15% refused to follow the procedures. Most of accidents were reported in central zone of Córdoba and the lower limbs were the main areas of the bites. It was also observed that canines are the primary responsible for contact (94.8%). With respect to control actions, in Córdoba 353 PEP were registered (100 more than in 2017).

The increase observed in number of samples, bitten people and PEP, was probably due to surveillance system improvements carried out. However, other issues continue representing a system weakness, such as bitten people who refuse to follow the procedures, underreporting of people who do not have information or access to health system and the high number of stray dogs. It is necessary to give special attention to rabies as an important emerging and re-emerging disease. Consequently, the surveillance of Rabies in Córdoba should continue to intensify the prevention and action measures in order to achieve the control of this zoonoses.

EPIDEMIOLOGICAL SITUATION OF ANIMAL RABIES IN BUENOS AIRES IN THE LAST 10 YEARS

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Presenter: María Lorena Vico

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Introduction. Rabies is a zoonotic disease caused by the rabies virus, of the Lyssavirus genus, within the family Rhabdoviridae. Since 1977 a Provincial Rabies Preventive Plan reverses the current status of the disease through health education programs, epidemiological surveillance and domestic animal vaccination coverage. Last human rabies case in this region was recorded in 1981, and also since 1984 there have been no animal cases for this virus antigenic variant.

Objective. The aim of this study is to evaluate the epidemiological current situation of our region through the data of the samples submitted for rabies diagnostic to our Laboratory, in the period between 2008 and 2018.

Materials and Methods. A total of 9900 brain samples from animals were sent from the Zoonoses health centers from different states of Buenos Aires to our laboratory between January 1st of 2008 and December 31st of 2018. The major amount of samples tested were brain tissue of dogs, domestic cats and insectivorous bats.

Two tests were performed for animal Rabies diagnostic: Direct immunofluorescence test (DFA) and intracerebral inoculation in mice with macerated brain. It was used a Nikon epifluorescence microscope with mercury lamp for DFA.

The statistic treatment of this data was by semi-annual percentage variation (VAP) of rabies rates using simple linear regression and nonparametric comparison tests of median between two periods (2008-2012 and 2013-2018) with the SPSS Statistics 21 program (IBM USA).

Results. From 3944 bat samples tested 203 were positive (5,1%). Statistical analysis did not throw significant difference in the periods of time studied. Seasonal variation analysis were not performed. Insectivorous bats are an important reservoir of the virus. Keeping good epidemiological surveillance levels by testing bat samples is an important way of disease monitoring in urban settlements.

SPATIAL ANALYSIS OF HUMAN EXPOSURE TO POTENTIAL RABIES VIRUS TRANSMITTERS IN COLOMBIA (2007-2016)

Marcela Rocío Arias Caicedo¹, Diego De Arruda Xavier¹, Catalina Alejandra Arias Caicedo³, Isis Abel Bezerra¹, Etiene Monteiro de Andrade², Elane de Araújo Saraiva¹

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²UFPA-PPGEAA Programa de Pós-Graduação em Estudos Antrópicos na Amazônia

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Presenter: Marcela Arias Caicedo

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In Colombia until the 1970s, human rabies occurred by dog variants. Between 2005 and 2006; Chocó department reported fourteen human deaths in indigenous population and tree in Afro-descendant communities, all by bat variants. After 2005, human deaths by rabies have been mainly caused by bat variant transmitted by bat and domestic cat. This study aims to analyze the demographic factors and geographical distribution of animal aggression in humans in Colombia between 2007 and 2016. It was realized using data of aggressions by animals transmitting of rabies notified in public health surveillance system of National Institute of Health. They reported 678.645 cases, occurring 76% in urban areas and in the male sex (54.7%). Women were more often aggressed by cats (61%). The most frequent age range was 5-9 years old (16.9%) and the most affected occupation was student (34%). For the interval from 20-24 years old, were registered the highest aggressions in farmer who had the main contact in mucosa or skin injured with biological material or secretions of bovine infected with rabies (49.3%) and health professionals who had the main contact in mucosa or skin injured with biological material or secretions of humans infected with rabies (73%). A low-high risk for aggression by all animal species was identified in Chocó department, showing the lowest incidence rates during all the years evaluated. The aggressions by dogs and cats and the contact with farm animals positive to rabies showed a high risk in Orinoquia and Andean regions, where human deaths transmitted by cats and bats were reported. Humans' deaths by dog aggression were reported in Atlantic region, area with historical human rabies transmitted by dogs, however with a low risk for dog aggression. Bat aggression is presented as high risk in the Vaupés department in Amazon region, mainly in indigenous population (98%).

EPIDEMIOLOGICAL PROFILE OF THE DIAGNOSIS OF ANIMAL RABIES IN FORTALEZA, CEARÁ - BRAZIL: WHAT CHALLENGES?

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Isadora Machado Texeira Lima, Francisco Barroso Pinto
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Presenter: Francisco Atualpa Soares Júnior
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According to WHO, Rabies is a fatal zoonotic viral disease present on all continents, transmitted to humans through contact (mainly bites and scratches) with infected animals, both domestic and wild. Domestic dogs are main responsible for rabies virus transmission to humans, being this one of the neglected tropical diseases that predominantly affects poor and vulnerable populations. In Brazil, the program of control and surveillance reduced the human cases significantly. On Ceará state, the capital Fortaleza had the last human case in 2002, after a intense program of vaccination on dogs and cats, twice on year. In the last 5 years, the Unity of Zoonosis Surveillance collect samples of brain tissues from suspected animals and send to the Rabies diagnosis laboratory.

	2014		2015		2016		2017		2018	
	+	-	+	-	+	-	+	-	+	-
Cão	0	186	0	151	0	102	0	125	0	74
Gato	0	27	0	26	0	20	0	30	0	6
Sagui	0	4	0	4	0	2	0	4	1	4
Morcego	0	5	0	10	2	9	1	15	0	13
Raposa	0	0	0	2	0	0	0	1	0	2
Bovino0	0	1	0	0	0	0	0	0	0	
Primatas	0	0	0	0	0	3	0	0	0	0
Cassaco	0	0	0	0	0	0	0	1	0	0
Total	0	222	1	193	2	133	1	175	1	99

The results shows that we must have two preoccupations in the control of rabies: 1 the wild animals, specially marmosets and bats; 2. Abandoned animals, specially cats, that can hunt bats for instinct. We estimate that around 100 thousands of cats are unrestricted, being potential hosts and transmitters of the disease.

ANIMAL RABIES IN TABULEIRO DO NORTE MUNICIPALITY - CE, HEALTH SURVEILLANCE ON ALERT

Rodrigues, VC¹; Lima, FMG¹; Moura, FBP¹; Chaves, CS¹; Maia, KM²; Lima, MFC¹

¹Secretaria da Saúde do Estado do Ceará/BR,

²Prefeitura Municipal de Tabuleiro do Norte/CE

Presenter: Vanuza Cosme Rodrigues

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Rabies is an infectious disease caused by viruses, it can attack animals and humans. Surveillance of animal disease is a public health concern, due to the risk of its transmission. In Brazil, animal rabies is endemic and detected in several animal species. The Northeast region still shows greater fragility to outbreaks or cases of rabies transmitted by dogs. The objective of this study is to describe the surveillance of animal rabies in Tabuleiro do Norte municipality - CE in 2017. Health surveillance professionals and health unit workers were trained to follow reports of rabies treatment in that municipality. So, continuous monitoring of aggressor animals was carried out, with the collection of samples for laboratory analysis of the animals that died. In addition, health education actions were carried out with the population in order to guide them about the prevention and risk of morbidity and mortality in humans. There were workshops in schools, meetings in the city council, participation in local radio programs, orientation in waiting rooms of Health Units, among others. Therefore, the residents informed suspect animals, to Municipal Health Department, for laboratory diagnosis. Then, 63 samples were analyzed: 01 dog, 02 cats, 59 bats and 01 fox; and were positive: 01 cat, 16 bats and 01 fox. Such positivity represented 25.57% of the samples collected. It should be added that municipality held 40.38% of the collections from the 10th Health Region of Limoeiro do Norte, which has 11 municipalities. Laboratory analysis is paramount for rabies surveillance. It should also be pointed out that permanent education for the population and health workers contributed significantly to identifying the circulation of rabies virus in the municipality and the continuous adoption of preventive actions.

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In Ceará State, rabies is an endemic zoonosis in urban and rural areas. The State Department of Health reported five deaths from the disease in the last ten years. Cases of the disease can occur when there are no routine preventive actions in health sector, strengthened by community engagement. Therefore, it is important to insert popular participation with collective construction of prevention and health promotion in coping with rabies. The present report aims to describe the popular participation in Quixeré / Ceará in the surveillance of the municipality's rabies. The research shows the work of mobilization and sensitization of the population for the passive surveillance of rabies, by the zoonosis sector of the Municipal Health Department (MHD). Initially, community health agents and endemic agents were trained as multipliers of prevention and surveillance information. School lectures and radio programs on rabies also encouraged the population to notify MHD about animals suspected of rabies, especially bats. Thus, from July 2015 to July 2018, 87 samples of rabies animals were sent, mainly from chiroptera (79), among feline (1), wild canid (4), nonhuman primate (2) and raccoon (1). A 238% increase in the number of samples analyzed from 2015 was observed compared to 2017. During the period, 23 animals were diagnosed with virus: 82.6% chiroptera and 8.6% wild canids. Popular participation in Quixeré left surveillance of rabies strengthened and attentive in the identification of suspect animals. Laboratory results allowed the identification of virus circulation and the risk of transmission of rabies in the municipality, as well as the continuous need for integration between the population and MHD in the prevention of the disease. It is therefore necessary to maintain education actions in the municipal territory as a stimulus to surveillance.

IMPORTANCE OF MARMOSETS, FOXES AND BITCHES IN RABIES SURVEILLANCE IN THE STATE OF CEARÁ – BRAZIL

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Isadora Machado Texeira Lima, Francisco Barroso Pinto**

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Ceará state is located in northeast of Brazil. It has a diverse fauna, with variable microclimates, favoring the proliferation of diverse animal species. In the historical series of human deaths from 1990 to 2003, of 40 deaths 33 (82.5%) had a history of contact with dogs. From 2004 to 2016 the profile began to change, with 6 deaths and only 1 dog involved (16.67%). The shift in this transmission chain began when there was massive investment in vaccination campaigns, elimination of unrestricted animals, education campaigns and social mobilization of the population, not only for the importance of dogs, but of the wild animals in the transmission. If we look at the historical series of 46 rabies deaths, we have 9 for marmosets (19.6%) and 3 for bats (6.5%). In recent years, surveillance of the program in the state has intensified compliance with the goal of vaccination of dogs and cats and intensified surveillance of suspicious samples, both wild and domestic. Thus, in 2016 of 425 samples sent, 61 were positive, being 39 bats, 1 sagui and 10 foxes. In 2017, 648 samples were analyzed, being 95 positive with 49 bats, 20 foxes and 4 marmosets. As of August 30, 2018, we have 416 samples analyzed with 21 bats, 15 foxes and 5 marmosets from 45 positive. Noting that not all municipalities send samples for structural and human limitations, it is noted that the rabies virus remains circulating in the wild, in the case of Ceará, with the viral types of wild bats, marmosets and canids in all regions of the State. The change in the circulation profile draws attention to the importance of population awareness and vaccination of domestic animals in order to avoid further human cases.

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Presenter: Fabíola Maria de Girão Lima
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In Brazil *Cerdocyon thous* is a wild reservoir of rabies virus. The Health Surveillance Secretary recorded 67 wild canids with virus in the Northeast region from 2015 to 2017. A domestic cat was isolated from the antigenic variant 2 (*C. thous*) in Ceará State in 2015. In 2017 Ceará State accounted 50% of the country's positivity. Thus, this work describes the model of passive rabies surveillance in *C. thous* in the municipalities of 10th Health Region (HR) - Limoeiro do Norte / CE / BR. At the time of virus isolation in these animals, there were 25 human attacks in the studied area from 2015 to July 2018. In addition, technical meetings to evaluate the actions of endemics and zoonoses led to monitoring of rabies in wild animals by sending samples for diagnosis. Surveillance at *C. thous* is done by collecting dead animals on the roads and those sacrificed during the attack on victims. Also, a surveillance network counts on zoonosis professionals, epidemiology, health care and population. Passive surveillance in *C. thous* has been stimulated, revealing increase in cases. From 2015 to July / 2018, 32 samples were sent for examination. 46.8% of the animals were diagnosed as positive in 36.6% of the municipalities with Jaguaribe getting 46.6% of the samples. Health surveillance is more attentive to the prophylaxis and monitoring of areas with viral circulation. Thus, the study shows the effect of sensitization on the importance of passive rabies surveillance in *C. thous*, at 10th HR, since the municipalities apprehended the need to monitor dead canids. This has contributed to risk management of rabies among the species, and in preventing disease in humans. It is important to strengthen the work in silent areas and to promote more information on wild rabies.

DEVELOPMENT OF A PREDICTIVE MODEL OF BOVINE RABIES OCCURRENCE IN PERU

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There is an important incidence of bovine rabies transmitted by the most common species of hematophagous bat, *Desmodus rotundus* in Latin America. In Peru, remains endemic despite control efforts, the disease still spreads insidiously. The present work aimed to develop a predictive qualitative model of the occurrence of bovine rabies in the 24 departments of the Peruvian territory.

The risk of rabies transmission from bats to livestock was estimated using decision-tree models of receptivity and vulnerability, established in previous works carried out in the South American region. Surveillance information related of bovine rabies outbreaks, bovine density, environmental changes, presence of bat roosts, and geomorphologic features of each district were obtained from official national databases and geographic information systems.

Several different scenarios were established using data from rabies outbreaks between 2012 and 2016, as well as certain quantitative values such as cattle density, forest remnants and terrain declivity that directly affected the establishment of receptivity and therefore of risk, were altered according to the type of distributions of these variables, in order to find the group of variables that allow to categorize the risk of each district and to be able to predict adequately the appearance of future cases of bovine rabies.

Most of the districts that presented bovine rabies cases in 2017 had been classified as high or moderate risk, which allowed an acceptable rate of success, so that the risk estimator for that year was classified as moderately accurate.

The model is dynamic, so it depends on information to improve its accuracy, which could allow targeting the efforts of the rabies surveillance and control services, with the adoption of control measures directed to the places of greater risk and the optimization of the deployment of field veterinary personnel throughout the country.

Taiana Andrade Freitas, Érika Dayane Leal Rodrigues, Francisco Amilton dos Santos Paiva, Armando de Souza Pereira, Taciana Fernandes Souza Barbosa Coelho, Alexandre do Rosário Casseb, Elizabeth Salbé Travassos da Rosa, Livia Medeiros Neves Casseb
Evandro Chagas Institute, Secretary of health surveillance, Ministry of health

Presenter: Livia Casseb

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Rabies is an anthroponosis characterized by acute viral encephalitis, the disease presents a severe clinical frame and is almost always lethal. Rabies is caused by Rabies lyssavirus, a virus that is kept and perpetuated in nature by mammals, which serve as reservoirs. Besides the disease being a public health problem, it is also a problem for the economy, because rabies in herbivores is responsible for causing great damage to livestock. Knowing the spatial-temporal distribution of rabies cases in the State of Pará is of paramount importance for control and prevention measures to be implemented. The objective of this study was to describe the prevalence of rabies in the State of Pará between the years 2000 and 2017. A descriptive epidemiological study was carried out, where a survey was carried out in the Evandro Chagas Institute of Rabies Diagnostic Laboratory database of samples received between 2000 and 2017 from the State of Pará. The positive cases for rabies and the total number of brain samples sent for diagnosis were distributed by species and year. Of the 16,693 samples received and analyzed in the laboratory, 1.87% (313/16,693) were positive. Of the 313 positive samples, 29.10% (94/323) were bovine, 26.08% (18/69) equine, 2.38% (13/545) feline, 1.74% (170/9,742) 0.29% (18/6,014) bats. In the analysis of the distribution of positive cases per year, it was verified that the year 2017 had a higher percentage of positivity, with 8.04%, followed by 2001 with 6.34% and 2000 with 6.19%. In conclusion, it was verified that animal rabies is endemic in Pará, with a variable occurrence between the years, with no pattern apparent cyclicality. The disease mainly affects cattle and horses. It is suggested to adopt measures of control and continuous surveillance of rabies in the State of Pará.

GENETIC CHARACTERIZATION OF CEPAS OF RABIES VIRUS, CIRCULANTS IN THE STATE OF PARÁ, BRAZIL, 2014-2017

Taiana Andrade Freitas, Érika Dayane Leal Rodrigues, Francisco Amilton dos Santos Paiva, Armando de Souza Pereira, Taciana Fernandes Souza Barbosa Coelho, Sandro Patroca da Silva, Samir Mansour Moraes Casseb, Livia Medeiros Neves Casseb, Elizabeth Salb  Travassos da Rosa
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The Rabies is an anthroozoonotic disease, of cosmopolitan distribution, caused by Rabies lyssavirus (RABV). The identification of viral variants makes it possible to know the origin of the circulating rabies virus in a given region and, consequently, the existing cycles and the species involved. Identifying and knowing the variants of RABV allows the recognition of issues associated with transmission and helps in the elaboration of measures of control and prevention of RABV. The objective of this work was to characterize genetically strains of the rabies virus from samples from the State of Par , received in the period from 2014 to 2017, in the Laboratory of Diagnosis of Rabies of the Evandro Chagas Institute (IEC). A total of 20 bovine (17/20), equine (2/20) and bats (1/20) samples were genetically characterized according to the methodology of Sanger et al (1977). The nucleotide sequences obtained were compared to each other and to sequences available from GenBank. The phylogenetic tree was constructed using Neighbor-Joining methods. According to the phylogenetic analysis, all the sequenced samples were grouped in the VAg3 group, with a bootstrap value of 99%, grouped into two clades, but it was not possible to observe territorial characteristics, such as groupings of variants by the mesoregion of the State. In the Clade I were grouped samples from the mesoregions of Southeast Paraense, Northeast Paraense, Southwest Paraense and Metropolitan of Bel m. In Clade II, samples from the southeast Paraense and Northeast Paraense were grouped. In the present work, it was observed that all the samples are related to samples characterized in previous studies carried out in the State of Par  and that the sequenced samples aligned with VAg3, where the results found in the State of Par  have been similar to those found in other States of Brazil and other Latin American countries, where the variant associated with *Desmodus rotundus* has been widely identified. The genetic characterization made possible a better understanding of the molecular epidemiology of the rabies virus in the State of Par .

RABIES DIAGNOSIS IN BATS FOUND IN URBAN AREAS OF SANTA CATARINA, BRAZIL, AND ITS IMPLICATIONS ON PUBLIC HEALTH

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Rabies is a zoonosis that causes acute encephalitis and has a high mortality rate. Bats, vampire or not, are one of the main reservoirs of the disease and show the symptomatology through behavioral deviations, resulting in death. The monitoring of viral presence in bats is indispensable in the current epidemiological picture of urban rabies, where more and more domestic animals end up contracting rabies through bats as a result of their synanthropic behavior. Therefore, viral monitoring in bats captured in urban areas makes possible to know the epidemiological status of the disease and, consequently, reduce the risks to public health caused by false evidence of the absence of viral circulation. This surveillance procedure is actually really important in Santa Catarina, a controlled area for urban rabies since 1981, and that since 1995 includes inspection of all suspicious bats collected in urban areas as the main surveillance method. In this context, 81 bats (non-hematophagous) collected from January to December 2017 in urban areas of Santa Catarina, were analyzed by real-time PCR. Of all the 81 bats, 4.93% (4/81) of the samples were identified as positive, distributed throughout northern, southern and Itajaí valley regions, attesting viral circulation. It should be noted that epidemiological surveillance influences the availability of resources designed to disease control and, besides that, referral of samples can act as a positive feedback by highlighting the need for greater investments in sector. Rabies epidemiology in bats should receive increasing attention from government institutions and researchers in order to provide effective control and prophylaxis strategies, avoiding cases of the disease in animals, and especially in humans.

DETERMINING THE POST-ELIMINATION LEVEL OF VACCINATION NEEDED TO PREVENT RE-ESTABLISHMENT OF DOG RABIES

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Human rabies annually causes approximately 59,000 deaths globally, with 98% of those deaths due to the canine rabies virus variant. The World Health Organization recommends that canine rabies endemic countries vaccinate 70% of their dogs each year to eliminate canine rabies. We estimated the cost-effectiveness of vaccination strategies to prevent post-elimination re-establishment of canine rabies.

We used the RabiesEcon transmission model to estimate the percentage of dogs that must be annually vaccinated to prevent re-establishment of canine rabies. We allowed for differences in transmission rates (varying the basic reproductive number), assumed risk of reintroduction (number and frequency of rabid dogs entering elimination area), ratio of susceptible dogs to humans (dog density) and dog life expectancy (turnover rates). Finally, we calculated the costs and health benefits of maintaining sufficient coverage over a 20-year time period.

Assuming 10 rabid dogs are introduced at week 1 into a canine variant rabies free area of 1 million human and 67,000 dogs, 45% of dogs must be vaccinated annually to prevent re-establishment of canine rabies. Continuing vaccination with 45% coverage can prevent approximately 60,000 rabid dogs and 19,000 human rabies deaths over a 20-year period. Average cost per rabies-related human death averted over a 20-year period was \$156. When there is a continuing risk of reintroduction every 5 years, 55% to 63% of dogs need to be annually vaccinated if 1 or 10 rabid dogs are reintroduced and initiated onward transmission, respectively. We found that the results were most sensitive to the assumed risk of re-introduction, transmission rates and dog turnover rates.

Following successful dog rabies elimination programs, public health officials should consider planning for the post-elimination risk of re-introduction. The estimates presented here will aid public health officials in implementing such plans.

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Ethiopia has long been among the most rabies-affected countries on the African continent with a national annual incidence rate of 12/100,000 population rabies exposures and 1.6/100,000 population rabies deaths. However, little information exists regarding the genetic diversity of RABV circulating in dogs or the existence of alternative rabies cycles maintained by other mammalian species. This work comprises the study of 230 sequences obtained from both wild and domestic animals collected throughout different regions of Ethiopia. Samples were collected by the Ethiopian Public Health Institute during the period 2010-2017. Partial N-genes from a subset of samples 187/230 (2012-2017) were sequenced de novo after being confirmed rabies positive by a real time RT-PCR assay (LN34). Complete N-gene sequences for 43/230 samples were obtained from previous collaborations with EPHI (2010). Sequences were compared against reference sequences representing major extant RABV variants across Africa. Results identified a dog rabies epizootic scenario throughout Ethiopia involving a seemingly homogeneous RABV variant that has been gradually spreading from an epicenter in the Oromia region. No distinct geographical pockets of dog rabies variants were identified in the phylogenetic analysis, suggesting dog rabies spreads across regions with no apparent boundaries. A 3.3% divergent RABV variant circulating in side-stripped jackals of the SNNP region was identified, which appears to constitute an independent RABV cycle. RABVs associated with Ethiopian wolves were consistent with the dog epizootic RABV variant, which suggests this endangered species has been recurrently affected by the dog rabies epizootic. There was no evidence of dog-maintained rabies imported from other African countries indicating Ethiopia has its own dog maintained and wildlife-maintained RABV variants, both of which are part of the Africa 1a clade. This snapshot of rabies dynamics in Ethiopia provides important baseline data to monitor progress on prevention and control efforts in Ethiopia.

LANDSCAPE GENETICS OF SMALL INDIAN MONGOOSE IN PUERTO RICO: A PRELIMINARY ANALYSIS

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The small Indian mongoose (*Herpestes auropunctatus*) in Puerto Rico is an invasive species and rabies reservoir. Rabies control programs can benefit from understanding host population connectivity across areas of management interest. Landscape genetics can inform population connectivity, but its utility depends on sufficient sampling to detect genetic population structure associated with identifiable landscape features. We collected samples from different habitats on opposite sides of Puerto Rico. We analyzed 65 samples from a 100 ha region of EL Yunque National Forest in northeastern Puerto Rico. We also analyzed 109 samples from the western side collected from a 2000 ha area; 93 were from Cabo Rojo National Wildlife Refuge and 16 were from the Pitahaya region of the Boqueron State Forest. We used eight microsatellite loci, specific for mongoose, to genotype all samples. Allele frequencies were analyzed using a Bayesian analysis of population structure, with and without GPS location information added a priori. Without a priori location information, individuals grouped into 16 clusters. One cluster was only located on the eastern side and four of the clusters were only on the western side; the remaining clusters were shared across sampling areas. With a priori location information, the number of clusters was reduced to three. For the eastern side, 2%, 3%, and 95% of the individuals were in clusters 1, 2, and 3 respectively. On the western side, 10%, 77% and 13% of individuals were in clusters 1, 2, and 3 respectively. Our results suggest some allelic diversity is shared but some may be unique to regions of the island. However, this is an ongoing study and further representative sampling is needed to fully understand population structure of this invasive species and potentially help define management units for rabies control.

LABORATORY DIAGNOSIS OF RABIES IN WILDLIFE ANIMALS

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Rabies is an acute and progressive encephalitis caused by a single-stranded RNA virus belonging to the family Rhabdoviridae, genus Lyssavirus and species Rabies lyssavirus (RABV). Considering the decrease in the number of rabies cases in dogs and cats and the maintenance of control measures against rabies in herbivores, wild animals have been highlighted as important reservoirs of this disease. Besides that, it is worth noting the increased risk to humans due to synanthropic habits acquired by wild animals. The objective of this work was to conduct a retrospective study on the occurrence of rabies in wild animals forwarded to the diagnosis of the reference laboratory of the State of São Paulo. For this, the databases of the reference laboratory were used and the samples received in the period from 1996 to 2003, the result obtained by standard direct immunofluorescence technique were used for the samples of different wild animal groups from the State of São Paulo. In this period, the total number of samples received was 10,228, and the animal group with the highest number of recorded was the Chiroptera, totaling 9932 samples (97.11%), of which 206 (2.7%) were positive for rabies. The second group was wild canids with 296 (2.89%) samples and 2 (0.68%) positive samples. The highest percentage of samples received was from Chiroptera and for both groups of wild animals evaluated, the number of positive samples for rabies was low. Considering the analyzed period and the samples evaluated, the circulation of RABV in wild animals is evident, but with low occurrence of positivity. These results highlight the importance of sending wild animals to laboratory diagnosis of rabies. Further studies should be carried out to understand the maintenance of RABV in these species.

RABIES CAUSED BY WILD CANIDS IN RIO GRANDE DO NORTE, BRAZIL: A PERCEPTION OF TRANSMISSION

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The rabies (RABV) in wild canids (CS) in the Northeast of Brazil, suggests a cycle of the disease of great epidemiological importance in the last decades, given the frequency of CS confirmed, affecting domestic dogs/cats. The objective was to understand the perception of human exposures, dogs/cats, regarding the transmission of rabies by CS, in June/2018. The qualitative-descriptive research, through the application of a semi-structured questionnaire and convenience sampling of 151 volunteer residents, in the municipalities of Extremoz and Caicó with rabies registries in CS in 2016/2017. Responses on knowledge of transmission by CS: 63.8% of the interviewees affirmed a possible transmission to humans and 57.1% for dogs/cats, by means of a bite (89.5%). About previous knowledge of rabies: 63.8% knew of the fatality, 64.8% were informed by the media (Radio/TV), they had doubts in the form of transmission and prevention. Regarding the presence of CS with RABV:15.4% observed animals with suggestive symptoms and 22.1% observed dead or ran over, without rigging the zoonoses service. Regarding the need for vaccination of dogs/cats: 77% affirmative. About human prophylaxis: approximately 59.6% did not seek care and 82.4% were unaware of the risk of becoming ill. Changes in the environment, such as the burned 59.6% contributed to the invasion of CS observed by flipping trash and fetching water. As for creating CS as PET: 92.2% showed no interest. The reality Caicó and Extremoz, show previous knowledge about the transmission of rabies by CS, it is noteworthy that social conditions related to environmental issues and synanthropic habits suggest the maintenance and perpetuation of rabies in the region. The lack of interest in creating CS as pets does not prevent the approach of dogs / cats. We suggest underreporting of cases and the need for implementation in educational and surveillance actions on RABV transmission by CS.

DETECTION OF RABIES VIRUS IN NON-HUMAN PRIMATES DURING YELLOW FEVER EPIZOOTICS IN BRAZIL, 2016 AND 2017

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In recent years, the participation of NHP in the wild cycle of rabies in the northeast region of Brazil has been registered, with the specific rabies virus *Callithrix jacchus* (AgVC) responsible for human and marmoset deaths. However, among the other diseases that affect NHPs, Yellow Fever (YF) has an important impact. The objective of this work was to describe the results of biological collections of material for the detection of rabies, during the monitoring of the epizootics by the Yellow Fever recently registered in Brazil. A descriptive study of the Brazilian territory was carried out between the years of 2016 and 2017, based on reports of epizootics in NHP received by the Ministry of Health, in the National System of Notifiable Diseases; Laboratory Environment Management and Standardized Worksheets. 1,800 NHP samples were processed for rabies research, and the year of 2017 (N 1717) had an increase of ~ 20 times compared to 2016 (N 83). Amongst the total number of NHP samples, 08 were positive for rabies, four of which AgVC had been already registered in different cities of the State of Ceará, and four were isolated from rabies bats (AgVMH) from Bahia and Espírito Santo, undescribed in NHP until recently. Due to the epizootics of NHP by YF, an increase in the frequency of notification, collection and sending of samples for the diagnosis of rabies in these animals was observed. The detection of RABV in NHPs with differentiated variants was important to verify the circulation of AgVMH in NHPs, even in places without previous records. Therefore, the epitope event of YF in NHPs has been shown to be an opportunity to increase detection of rabies in NHP, including unregistered region and variant, to date. These are the benefits of integrated surveillance of zoonoses and their contribution to public health.

COMPARISON OF THREE DRUG COMBINATIONS FOR RACCOON IMMOBILIZATION

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Raccoons (*Procyon lotor*) are a rabies reservoir and require handling for evaluating rabies management and conducting rabies research. Annually, over 5000 raccoons are immobilized for sampling in the US as part of rabies management activities. Safe handling of these animals in the field not only requires drug combinations that provide effective and predictable results with high safety margins for both the animal and personnel handling the animal, but also have a low probability of abuse. United States Drug Enforcement Administration (DEA) scheduling relates to the probability of a drug being abused, with class I drugs associated with the greatest potential for abuse. We compared three drug combinations; two of which were previously untested in raccoons: butorphanol-azaperone-medetomidine (BAM; class IV) and nalbuphine-azaperone-medetomidine (NAM; unscheduled). The third combination, ketamine-xylazine (KX; class III) is commonly used. Through a dose titration process, we identified optimal drug dosages of 0.016 mL/kg for BAM, 0.018 mL/kg for NAM, and 0.096 mL/kg for KX. The induction time was similar for all drugs, but only with KX were raccoons able to recover unaided by reversal drugs. After giving reversals, recovery times for BAM and NAM were relatively quick (average recovery less than seven minutes). Based on blood oxygen saturation levels and respiratory rate, oxygen was administered to 72%, 67%, and 17% of the raccoons immobilized with BAM, NAM, and KX, respectively. Breathing was cyclic in raccoons immobilized with BAM and NAM, and some raccoons were given reversal agents prior to completing a workup due to low respiratory rate or low oxygen saturation levels. Raccoons immobilized with KX were observed with a more regular breathing pattern. Based on our results, it is highly recommended that both oxygen and associated reversals be available when using BAM or NAM to immobilize raccoons.

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Considered a serious public health problem, rabies is a zoonosis that presents a great diversity of reservoirs, increasing the circulation of the virus, being important the surveillance and constant sending of animal samples for the traceability of the disease in endemic areas. The state of Ceará presents a historical importance with cases of rabies in humans, great animal positivity, highlighting the importance of wild animals in this cycle. The objective of the study was to distribute the samples of positive and positive samples from the Laboratory of the Central Public Health Laboratory (LACEN-CE) through the direct immunofluorescence technique (IFD). Of the 184 municipalities, 102 (55.4%) sent samples that included the following animals: dog, cat, fox, bat, raccoon, sagui, bovine, sign, equine, goat and sheep. Among the municipalities that sent samples, 46 (45%) had positive samples for rabies during this study period. In the period from 2013 to 2016, there were 161 positive samples received by the rabies diagnostic laboratory, highlighting the municipalities: Aurora (24), Tabuleiro do Norte (17), Jaguaribe (11), and Limoeiro do Norte (9). Rabies is a zoonosis belonging to the list of diseases of mandatory notification and requires monitoring and constant monitoring of viral circulation, carried out by continuous sending of samples for laboratory examination. The occurrence of wild animals with a positive diagnosis for rabies has been increasing over time, emphasizing the importance of strengthening active and passive surveillance of rabies in the field-laboratory environment, sending samples of sentinel animals, such as suspect animals, responsible for cases of aggression, run over, those who have behavioral changes and neurological diseases or death to clarify.

DEVELOPMENT AND VALIDATION OF A COMPETITIVE MULTI-SPECIES ELISA FOR ANTI-RABIES VIRUS ANTIBODIES QUANTIFICATION

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Rabies is a zoonosis where human mortality is still an important issue worldwide. The generation of rabies virus neutralizing antibodies (RVNA) is vital to develop an effective vaccination schedule. Detection and quantification of anti-rabies antibodies plays a central role in rabies control. Currently, two RVNA quantification tests are available to measure RVNA, requiring the use of animals and rabies virus manipulation. As an alternative to these in vivo assays, a commercial ELISA kit (PlateliaTM RABIES II) is available, but due to its high cost, it is not affordable to regional surveillance laboratories.

This work presents the validation of a competitive ELISA (c-ELISA) that allows the quantitation of RVNA in multi-species sera. Thus, this assay consists in the competition between the RVNA present in serum and a biotinylated-rabies virus monoclonal antibody. Purified Rabies Virus-Like Particles (RV-VLPs) were used as capture antigen. WHO second International Standard for anti-rabies immunoglobulin was employed as a reference.

The log-transformed titer was plotted against the OD_{492nm} signal, obtaining a lineal model. The correlation coefficient was -0.98 and a F-test was applied, establishing that titers between 0.75 to 0.09 IU/mL adjusted to a lineal model (p: 0,28).

The accuracy was determined using spike samples. Applying standards fortified into matrix real samples, recoveries of $105 \pm 3 \%$ were obtained. In addition, the neutralizing titers samples were determined using the PlateliaTM kit as a reference method and a t-test was applied to assessing the c-ELISA accuracy. The assay precision was determined by the intra and inter-assay precision (CV%), being $5 \pm 3\%$ and 10%, respectively.

Although, the reproducibility and robustness of the c-ELISA must be determined, the methodology described here demonstrates that this assay is a reliable, accurate and precise method for the neutralizing antibodies quantitation in multi-species sera, which can replace the already available tests.

IMMUNOHISTOCHEMISTRY FOR DIAGNOSTIC OF RABIES BY USING A NEW MONOCLONAL ANTIBODY

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Rabies disease is an important zoonosis with impact in animal production. Immunohistochemistry (IHC) has been successfully tested for diagnostic on formalin fixed samples, but the primary antibody may not be easily available in many countries. The aim of this study was to evaluate the performance of a new monoclonal antibody (Mab) locally developed, for the detection of rabies virus in naturally infected cows by IHC. Sections (3 µm in thickness) of formalin fixed- paraffin embedded samples of stem brain from 10 cows positive for Rabies by direct immunofluorescence and biological test (positive samples) were tested. Additionally, 10 samples of stem brain from healthy cows coming from areas where the vector of the disease is not present (negative samples) were also analyzed. After deparaffinized, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol. Antigen retrieval was performed by humid heat treatment (121°C, 15 min) in citrate buffer (monohydrate citrate, 10 mM, pH 6). A blocking step was performed (BSA 1% in TBST), and the anti-Rabies Mab diluted 1/1000 in TBST was added and incubated at 4°C overnight. The reaction was revealed using the LSAB2 system and AEC (DAKO Cytomation System). All rabies samples showed intracytoplasmic immunostaining in neurons from different areas of parenchyma, while negative samples stayed totally clear. Unwanted background was absent. Immunohistochemistry recognized all positive samples and was negative for all control cows. The quantity of cases analysed did not allowed estimate sensitivity and specificity of the tested assay, but the correlation observed between IF and IHC for positive and negative samples suggested that accuracy of the test might be good. Obtained results indicated that the tested antibody can detect the Rabies Virus on formalin fixed tissue samples. Thus, immunohistochemistry can complement other confirmatory tests when those can not be performed.

DEVELOPMENT OF REAL-TIME RT-PCR (RT-QPCR) IN SAMPLES OF RABIES VIRUS (RABLV) GENETIC LINEAGE COMPATIBLE WITH CANIDS (AGV2)

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Despite the decline in the number of cases of human rabies transmitted by dogs in Brazil, there are still records of occurrence, mainly in north and northeast Regions. Between 2011-2016, six cases of human rabies were diagnosed with the genetic lineage of canids. This study aimed to develop a real time RT-PCR (RT-qPCR) in samples of rabies virus (RABLV) genetic lineage compatible with antigenic variant characteristic of canids (AgV2). Seventeen samples were selected from central nervous system of mice inoculated with AgV2 RABLV. Total RNA extraction was performed with TRIzol, and reverse transcription followed by polymerase chain reaction (RT-PCR) using specific primers for the N gene. The amplicons were purified and sequenced using the same primers used in RT-PCR. For the development of RT-qPCR to AgV2 samples, genetic sequences were aligned and a preserved and common area was chosen. Using the software Primer Express the most appropriate place for the synthesis of primers and probe was defined. From cDNA RT-qPCR was performed using the primers and specific probe designed for AgV2. A sensitivity test was performed for both techniques used and Fisher's exact test ($p > 0.05$) was used for the proportion of positives. All samples were tested positive in RT-PCR, while in RT-qPCR, 15 (88.2%) were positive and two negative, resulting in a diagnostic sensitivity of 89.5% RT-qPCR. The comparison of RT-PCR and RT-qPCR made by Fisher exact test as to the proportion of positives detected was not statistically significant ($p=0.48$). The RT-qPCR developed in this study was efficient in detecting the rabies virus in AgV2 samples with the advantage of being a faster technique that makes possible the early identification of positive samples without the need of DNA sequencing, providing a fast decision-making thereby leading an effective epidemiological control of this important zoonosis.

COMPARATIVE PERFORMANCE STUDY OF TWO RNA EXTRACTION METHODS BY THE RT-PCR AND RT-QPCR TECHNIQUES FROM BOVINE CENTRAL NERVE SYSTEM (CNS) SAMPLES NATURALLY INFECTED BY RABIES VIRUS (RABV)

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The detection of the RABV by the direct fluorescent antibody (DFA) may have varying results, once that the virus does not infect CNS structures uniformly. For this reason, the development and implementation of alternative methodologies, such as conventional RT-PCR and RT-qPCR, became important for the diagnosis. RNA extraction is also an essential factor influencing the sensitivity of molecular techniques for the detection of RABV RNA as a diagnostic test. These assays are usually hampered by the low virus titer found and PCR inhibition due to reagent residues or other unknown factors during RNA extraction. Rabid bovine CNS samples were analyzed by RT-PCR and RT-qPCR comparing RNA extracted with the guanidinium thiocyanate (TRIzol reagent) and commercial RNA extraction silica column (RNAspin mini isolation kit, GE Healthcare) during the period of March 2015 and April 2016. 186 CNS samples of 40 bovines were found as positive by DFA were included in this study. Extracts prepared using the silica column RNAspin mini isolation kit yielded the most consistent results by RT-qPCR and RT-PCR. The TRIzol reagent failed to confirm 52 RABV DFA-positive specimens by RT-qPCR. On the other hand, there were only two discordant results tested by RT-qPCR in DFA-positive extracts produced with RNAspin mini isolation kit. RABV nucleic acid was detected by RT-PCR in 161 of 186 (86.5%) DFA-positive samples that were manually extracted with TRIzol reagent, while viral RNA was detected in 168 of 186 (90.3%) DFA-positive extracts prepared by the RNAspin mini isolation kit. According to the results, the difference of the detection between those two extraction techniques demonstrates amplification problems of the nucleic acids in the samples extracted with TRIzol reagent, suggesting a higher efficiency of the extraction with the RNAspin mini isolation kit.

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EVALUATION OF POLYCLONAL ANTI-RABIES VIRUS IGG ANTIBODIES FOR RABIES DIAGNOSIS BY INDIRECT RAPID IMMUNOHISTOCHEMICAL TEST

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Presently the gold standard diagnostic technique for rabies is the direct immunofluorescence assay which is very expensive and requires a high level of expertise. As an alternative, the rapid immunohistochemistry technique has been promise to be simple and cost effective diagnostic tool for rabies and can be performed on field condicions prevalent in developing countries. Here, we evaluated the polyclonal anti-rabies virus IgG for Rabies lyssavirus (RABLV) detection by indirect rapid immunohistochemical test (IRIT). For this, horse hyperimmune serum against purified rabies virus was used for purification of the polyclonal IgG by ionic exchange chromatography on QAE sephadex A-50 (GE Healthcare) followed by HiTrap protein-A column, 5mL (GE Healthcare). The purity and affinity of IgG obtained were analyzed by 10% SDS-PAGE (under reducing and non-reducing conditions) and indirect ELISA, respectively. The purified IgG concentration was estimated by methods absorbance at 280 nm. The specificity and sensibility of anti-RBVL IgG were tested by IRIT in positive (n=20) and negative (n=20) CNS samples for rabies of different species animal (bovine, cat, dog, equine and bat). As results, the purified IgG contained one band of molecular weights ranged from 250 to 150 kDa under non-reducing conditions, and two bands, one at approximately of 52-58 kDa (H-chain) and another band at 22-29 kDa (L-chain) under reducing conditions, showing electrophoretic pattner compatible with horse IgG. In addition, it had high-affinity virus rabies protein recognition by indirect ELISA. The analyses of samples by IRIT revealed that the purified IgG obtained 100% of diagnostic specificity and sensibility for RABLV detection. In conclusion, our results demonstrate that the polyclonal anti-rabies virus IgG may be used as a diagnostic reagent for rabies using IRIT, with the expectation of to raise of availability and to cost reduction of the epidemiological surveillance to developing countries.

EVALUATION OF 6 TAQMAN RT-RTPCR KITS ON 2 THERMOCYCLERS FOR THE RELIABLE DETECTION OF RABIES VIRUS RNA

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Anses Nancy laboratory for rabies and wildlife

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Rabies is diagnosed in animals postmortem with primary diagnostic tests prescribed by the World Organization for Animal Health, such as the fluorescent antibody test, the direct rapid immunohistochemistry test, or pan-lyssavirus polymerase chain reaction (PCR) assays. The development of various real-time (rt) RT-PCR methods, nucleic acid extraction robots and rtPCR platforms has resulted today in a large arsenal of high throughput, robust and reliable rtPCR assays for rapid and accurate detection of lyssaviruses.

We evaluated the performance of 6 Taqman RT-rtPCR kits routinely used in National Reference Laboratories participating in interlaboratory tests for rabies in order to identify those that producing best results for RABV detection in terms of sensitivity and repeatability. The study was undertaken on a synthetic CVS-RNA with 2 real-time thermocyclers showing different throughput capacities. We showed that changing the master mix overall did not influence the Taqman RT-rtPCR performance, regardless of the thermocycler used. The limit of detection at the 95% confidence level was 18.1 to 25.8 copies/ μ L and 16.7 to 21.5 for the thermocyclers Rotor Gene QMDx and Mx3005P, respectively. Excellent repeatability was demonstrated for CVS-RNA samples of 100, 50, and 25 copies/ μ L, regardless of the thermocycler used. RABV field samples (n = 35) isolated worldwide gave positive results using the most efficient of the 6 kits tested, with a copy number of 6.03×10^2 to 6.78×10^7 RNA copies per reaction. The data accumulated in the study underline the combined sensitivity and robustness of the Taqman assay, for a sensitive and rapid detection of rabies virus RNA.

DEVELOPMENT OF MOLECULAR DIAGNOSTIC AND TYPING TOOLS FOR RABIES VIRUSES OF PERU

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This study sought to develop molecular tools for detection and typing of Peruvian rabies viruses (RABVs) with the principal focus on those associated with vampire bats, the predominant variant currently circulating in the country. RABV N gene sequences from a previous Peruvian study, that had identified three distinct vampire bat-associated lineages (L1-L3), were used together with other data to identify targets useful for development of a RT hemi-nested PCR (RT-hnPCR) for virus detection. In addition three forward sense lineage-specific primers were designed which, when used with a common reverse primer, should generate products of different sizes readily differentiated by gel electrophoresis. To evaluate these tools suspensions of 45 brain samples of bovine or equine origin from across Peru, all of which had been diagnosed as rabies-positive by the direct fluorescent antibody test at SENASA, were applied to FTA cards and transferred to the rabies laboratory in Ottawa, Canada. Total RNA was extracted from the cards using a QIAgen® RNeasy kit with a modified protocol and evaluated by the diagnostic RT-hnPCR. Due to the limited amount of tissue recovered, detection of viral RNA required two rounds of PCR and was successful for all samples except one. An optimised second round PCR employing the lineage-specific primers differentiated the 44 PCR-positive samples among the three Peruvian lineages. The accuracy of these lineage assignments was confirmed by sequencing of amplicons through the region targeted by these primers and phylogenetic comparison to Peruvian isolates previously sequenced. A single equine sample, originally identified as a member of L2, was an outgroup in the phylogeny; BLAST analysis confirmed that this isolate matched the Bolivian dog variant. Accordingly a dog-variant specific primer is currently being evaluated. A real-time RT-PCR, previously developed to detect all rabies viruses circulating globally, detected the virus in all 45 samples.

REVERSE TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) FOR RABIES VIRUS ISOLATED IN VIETNAM

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RT-LAMP is fast, easy, simple, sensitive and specific diagnosis and could be completed within 1 h and conducted isothermally with a conventional water bath or heat blocks. This method is ideal for both in the field of remote area and point of care testing in the hospital. Recent human rabies in Vietnam were mainly in northern and central northern mountains provinces. In 2015, 78 rabies patients were reported throughout the country. There were more than 100 rabid dogs, annually. Rabies viruses (RABV) were genetically classified into two groups of Group 1 and 2, and viruses of Group 1 was separated into subgroups of 1A and 1B (Jpn.J.Infect.Dis. 64:391-396, 2011). Distribution of Group 1B and 2 is in the northern provinces, and Group 1A is in the southern provinces. Laboratory diagnosis of rabies patients and animals is basis for surveillance and understanding of epidemiology in Vietnam. RT-LAMP focused on RABV in Vietnam was developed by redesigning of primers for RABV in the Philippines (Jpn.J.Infect.Dis. 62: 187-191, 2009). RNAs extracted from saliva and cerebrospinal fluid of patients and brain of dogs were examined, and DNA products were detected as DNA ladders by agarose gel electrophoresis and simply visualized with fluorescent dye by ultraviolet light. Total 11 primers of two outer-forward, three inner-forward, three inner-backward, two loop and two outer-backward successfully amplified RNA of RABV classified into three groups in Vietnam. The detection limit of RT-LAMP was 20 pfu of virus. The sensitivity and specificity of RT-LAMP were 100 % and 96 % compared to RT-PCR (Virology 194:70-81, 1993 and J.Virol.Method 81:63-69, 1999), respectively. RT-LAMP is useful for capacity building of rabies laboratory in remote area and strengthening of rabies network to support an action plan for rabies control and elimination in Vietnam.

DEVELOPMENT AND PRODUCTION OF A RECOMBINANT MINI-ANTIBODY AGAINST RABIES VIRUS

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Rabies is the zoonotic infectious disease with the highest case-fatality rate. Rabies diagnostics rely on laboratory testing and make use of specific antibodies. Several rabies virus (RABV) neutralizing mouse-derived monoclonal antibodies (mAbs) have been identified for this purpose. However, mAb production in mammalian cell-culture has a considerable cost, and the use of mice to obtain mAbs from ascitic fluid is increasingly restricted. In this scenario, novel biotechnological strategies are pursued to improve the availability of these valuable diagnostic tools. We have developed a specific recombinant mini-antibody against RABV formed by the variable region of the heavy and light chains of a previously described neutralizing mAb (CR57) reactive against epitope 1 of the viral glycoprotein G (RV-GP). Both light and heavy chains variable regions of CR57 were covalently linked by a flexible peptide linker and fused to the maltose binding protein (MBP) to improve the solubility of the polypeptide. Expression of MBP-scFv57 was optimized using an E.coli expression system. The recombinant mini-antibody was recovered from the soluble fraction and purified using a maltose-affinity column. Yield was 75g/l of initial culture. The integrity and stability of the purified MBP-scFv57 were verified by SDS-PAGE/western blot, confirming that MBP fusion also improved the stability of the recombinant mini-antibody. The purified mini-antibody recognized RV-GP in the membrane of transiently-transfected HEK-293 cells. Our preliminary results show that this mini-antibody is efficiently produced as a soluble and stable protein in bacteria and can be affinity-purified in one step, preserving its binding activity. This mini-antibody is meant to be used as an inexpensive reagent capable of detecting the presence of virus in tissue samples or infected cells.

DETECTION OF ADVENTITIOUS AGENTS IN RABIES VIRUS MASTER SEED IN CELL CULTURES

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Manufacturers of biopharmaceutical products are required to ensure that their products are safe for use in humans and animals. A major concern for many biological materials is viral contamination. Conventional adventitious virus testing in cell cultures have been applied as standard methods to exclude the presence of extraneous viruses in biological raw materials, cell culture substrates, viral seeds, and virus harvests used for biological medicinal products and vaccine manufacturing.

The Adventitious laboratory in INTA have the facilities and procedures needed to perform the Adventitious agents control in biological products. The test schemes include in vitro tests in cell cultures or embryonated chicken eggs and in vivo tests in adult and suckling mice.

In particularly, for Rabies Virus Master seed (MS), the sample is normally tested by inoculation into different cell cultures, including Vero cells, cells of the species for which the vaccine is recommended (human, canine, feline) and cells of the species for which the MS was isolated and/or propagated (bovine, porcine, rodent). As the Rabies Virus MS is capable of replicating in some cell in which it is to be tested, the MS must be neutralized prior to inoculating indicator cells.

The cells are incubated at 37°C and observed for a period of 14-21 days, and it is considered "not-detected" if none of the cultures shows evidence for the presence of any extraneous agent, verified by microscopic evaluation throughout the observation period and at the end of the observation period by test for haemadsorbing viruses and for specific extraneous agents from bovine, porcine, feline and canine species by immunofluorescence detection.

MS and cell lines used to produce Rabies Virus vaccine must be tested using a broad range screening assay to detect any potential contamination caused by unspecified agents to ensure they are safe of adventitious agents.

RABIES SEROLOGY PROFICIENCY TESTING: IMPROVEMENTS FOR COMBINING INTERNATIONAL STANDARD REQUIREMENTS AND STATISTICS FOR ASSESSING THE LABORATORIES

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Domestic carnivores can introduce rabies into disease-free countries or areas if they are incubating the disease and transported during the pre-symptomatic period. Quarantine was imposed in many countries to prevent such introduction. 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 known to be 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.

To guarantee an effective control system of the laboratories willing to carry out the rabies serological controls, the European Commission decided to establish a system of community approval of such laboratories. As the specific institute to coordinate the approval of the laboratories, designated by the European Commission in 2000, our main task is to organize annual rabies serology proficiency tests for laboratories already agreed or willing to be agreed to perform rabies serological controls in the frame of international trade of pets. Our laboratory has organized them since 1999. Over the years, the network has increased to reach 77 worldwide participants in the April 2018 rabies serology proficiency test campaign.

To guarantee a reliable way of performances' assessment to participants and to have recognition of our work performed for many years, we have improved our process to comply with both standards ISO/CEI 17043:2010 (General requirements for the competence of the proficiency testing providers and for the development and carrying out of proficiency testing programs) and ISO 13528:2015 (Statistical methods for use in proficiency testing by interlaboratory comparison). The improvements performed to comply with the ISO/CEI 17043 Standard and the statistical approach used for analyzing participants' data, based on the use of compiled previous data, will be presented.

GENETIC IDENTIFICATION OF BATS SENDING TO RABIES DIAGNOSIS: SEQUENCING PARTIAL OF CYTOCHROME C GENE OF MITOCHONDRIAL DNA

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Bats are recognized as an important reservoir of rabies virus, nowadays they are responsible for most cases of human rabies in Americas and play a leading role in transmission of rabies in Latin America. The correct identification of species that act as reservoirs is important for surveillance measures and for comprehension the dynamic of the diseases in these animals. The morphology identification of bats is simple and efficient but requires a trained professional and the integrity of carcass of animal. The purpose of this study was to perform the genetic identification through of the sequencing partial of cytochrome C gene of mitochondrial DNA (mtDNA COI). For this study were used lungs of bats, once Central Nervous System in most of cases is small and is intended for diagnosis of rabies. One hundred twenty eight samples of lung were submitted to DNA extraction, Polymerase Chain Reaction (PCR) with primers target COI gene and genetic sequencing. The nucleotide sequences were edited with CHROMAS (version 2.23 Copyright 1998–2004 Technelysium Pty Ltd.), and the final sequences were aligned using CLUSTAL/W in BIOEDIT version 7.1.3.016. The final sequences were used to confirm the identity of the species through Basic Local Alignment Search Tool (BLAST). In our study were identified nine species belonging to seven genera and three families: Phyllostomidae, Molossidae and Vespertilionidae. Rabies virus was detected in only one specie – *Molossus molossus*. The obtained results highlight that genetic sequencing partial of mtDNA COI was effective and lungs are an appropriated sample to isolation of mtDNA. In conclusion, sequencing of the mtDNA COI gene should therefore be used as an effective alternative to, or even in combination with, morphometric identification.

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Rabies is a fatal zoonosis, its etiological agent is a Lyssavirus that belongs to the Rhabdoviridae family of the Mononegavirus order. It is still a public health problem in our country so advances in diagnosis, prophylaxis and research on this disease are needed. The World Organization for Animal Health (OIE) recommends to reduce the use of laboratory animals. In this sense, the implementation of cell culture techniques results of major importance. The aim of this work was to evaluate and compare the susceptibility between neural and non neural cell lines to two different rabies virus strains. Neuroblastoma cells (Neuro-2A) and Baby Hamster Kidney cells (BHK-21) were cultured in DMEM-10 at 37° C in a 5% CO₂ humidified atmosphere. Two rabies virus fixed strains were used: challenge virus standard, CVS (107.46 LD₅₀/0.03ml) and Cg1 (108.29 LD₅₀/0.03ml). In both cases 20% W/V mice brain homogenates were diluted 1:5 (1 x 10⁻²). Afterwards, 1:10 serial dilutions were performed up to 1x 10⁻¹³. Cell lines were resuspended to 200,000 cell/ml and co-incubated with viral dilutions for 1 hour in agitation. For each dilution 6 wells were seeded in a 96-wells plate and incubated for 96 hours. After that, cells were fixed and stained with antinucleocapsid FITC-antibody. A whole well was considered positive with the presence of at least one cell with corpuscles. Detection limits (the dilution after which no infection is detected) were different for virus strains. Neuro-2A were more susceptible for virus infection with detection limits of 1x10⁻¹¹ for CVS and 1x10⁻¹² for Cg1 strain compared to BHK-21 cells with detection limits of 1x10⁻⁶ for CVS and 1x10⁻⁴ for Cg1 strain. This is a first attempt to use non neural cells in our laboratory with many potential applications such as vaccine production, diagnosis, cell adapted virus strains and investigation.

CHARACTERIZATION OF BATS TRANSMITTING OF RABIES VIRUS CAPTURED IN THE MUNICIPAL ENVIRONMENTAL PROTECTION AREA OF INHAMUM, CAXIAS-MA, BRAZIL

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The order Chiroptera (bats) is one of the most diversified groups of mammals in the world, with 18 families, 202 genera and 1120 species. This represents approximately 22% of the known species of mammals, which total 5.416 species being this number surpassed only by the group of rodents. For Brazil there are records of 68 genera and 174 species. Bats of the family Phyllostomidae are known as “leaf nosed bats” because they present a nasal appendix in the shape of a spear point, except for the representatives of the subfamily Desmodontinae, which has a horseshoe-shaped nasal leaf. The subfamily Desmodontinae comprises hematophagous bats, possessing three monospecific genera: *Diaemus youngii*, *Diphylla ecaudata* and *Desmodus rotundus* (the main transmitter of rabies in Latin America). The objective of this research was to morphologically characterize species of the subfamily Desmodontinae occurring in the Municipal Environmental Protection Area of the Inhamum (APA of the Inhamum), in order to subsidize relevant information on biodiversity in this area, and to investigate the presence or absence of the rabies virus in these species. Expeditions were made quarterly, each lasting six hours of catching effort, with exposure to six mist nets per night. The collected specimens were taken to the Laboratory, where they were photographed, sacrificed, labeled, weighed, measured and removed the brain to detect the presence of rabies virus in immunofluorescence tests. 26 specimens belonging to the subfamily Desmodontinae were recorded in the APA of the Inhamum, distributed in two genera and two species: *D. rotundus* with 25 specimens and *D. youngii* with one specimen. The morphological data evidenced the geographic expansion of these two species to eastern Maranhão. The rabies virus test was negative evidencing the absence of the rabies virus for these species and consequently the non-circulation of this virus in the APA of the Inhamum.

A CASE OF RABIES IN BATS (MAMMALIA: CHIROPTERA) IN A VERY POPULOUS AREA: A RISK FOR TRANSMISSION OF RABIES TO HUMANS

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The transmission of rabies to humans and domestic animals by hematophagous and non-hematophagous bats, directly and/or indirectly, is a fact found in the literature for different countries and regions of Brazil. In the last ten years, the state of Pernambuco was the scenario of two cases of human rabies caused by the hematophagous bat variant. The first one was transmitted by a hematophagous bat during feeding in a rural area; the second one was caused by a cat bite in an urban area of the metropolitan region of Recife, in which the victim died. In this work, a bat specimen (*Artibeus planirostris*) is recorded as positive for rabies, and the possibility of human rabies transmission is highlighted. This species is widely distributed in the northeastern region of Brazil and easily found in urban areas, foraging in search of both available fruit in the trees and shelter in the crowns thereof and in anthropic constructions, where they form small colonies. Reports of rabies in *Artibeus planirostris* in Brazil occurred in the southeast and northeast regions. The animal was found lying dead on the ground at 6:30 a.m., near a road with great circulation of people and animals such as dogs and cats in the Great Recife area, capital of Pernambuco, northeastern Brazil. The specimen in question was an adult animal, being positive for the biological and direct immunofluorescence tests. In the area, other specimens (1 *Artibeus planirostris* and 1 *Glossophaga soricina*) were found lying on the ground, being sent for analysis. This report shows that there is a great risk of rabies transmission by bats, mainly when they become ill and fall to the ground getting exposed in areas of great circulation of people and domestic animals, facilitating the contact between positive bats and humans or animals, especially if monitoring actions are not well developed.

Keywords: *Artibeus planirostris*, Frugivore; Lysavirus, Sinanthropic

BAT RABIES SURVEILLANCE IN THE STATE OF COAHUILA

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The high biodiversity that characterizes Mexico has allowed the presence of different enzootic cycles of rabies, also presenting critical geographical displacements of reservoir species, primarily by the intervention of the human being. In the state of Coahuila, 25 different species of insectivorous bats have been described. In this state the diagnosis of rabies began in 2010, generating an important sample bank and identifying 13 species of bats. Through a retrospective study of positive cases of rabies virus detected in bats, 5 cases were presented between 2013 and 2017 in Coahuila. These samples were submitted for molecular analysis to the Institute of Epidemiological Diagnosis and Reference (InDRE), the results indicated a 99% of similarity to rabies virus isolated from the following species of bats: in the municipality of Progreso (2017) with *Tadarida brasiliensis*, Piedras Negras (2016) with *Lasiurus Xanthinus*, Saltillo (2014) with *Lasiurus cinereus*, Cuatro Ciénegas (2014) with *T. brasiliensis*. The case of Piedras Negras of 2013 could not be characterized since the sample was not enough. In México, our laboratory is a pioneer in the taxonomic identification of bat species and their association with the different lineages of the circulating rabies virus. These initial contributions will allow for better knowledge and control of the virus, in addition to demonstrating the need for more efficient epidemiological surveillance in order to evaluate the circulation of the virus and the Chiroptera species. Although the presence of the hematophagous bat *Desmodus rotundus* has not yet been detected, its great ecological adaptation would allow the establishment of colonies, requiring the application of prevention and control measures to preserve human health and avoid economic losses, considering that one of the main economies at the north of the state is based primarily on livestock.

RABIES IN INSECTIVOROUS BAT IN THE CITY OF BUENOS AIRES

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The first bat tested positive for rabies virus (RABV) at the Instituto de Zoonosis Luis Pasteur (IZLP) was detected in 1965. It was found in the port of Buenos Aires and belonged to the species *Lasiurus ega argentinus*. After 26 years lacking positive results, in 1991 a specimen of *Tadarida brasiliensis* from the neighborhood of Barracas was tested positive, this had bitten a girl that was treated at the Hospital Carlos G. Durand. Since this episode, an increasing number of positive cases were found in different bat species frequently linked to contact or bites in pets and/or persons. Early diagnostics were conducted performing the Sellers' staining technique and mouse inoculation tests. Subsequently, Direct Fluorescence Antibody tests, RT-PCR, in vivo suckling mouse assay and cell culture were implemented. In addition, antigenic typing using a monoclonal antibody panel from the CDC (USA) and nucleotide characterization were implemented for strain identification. Between the first detection and June 30th, 2018, 4095 bats from Buenos Aires were tested for rabies, 128 among which were positive (prevalence: 3.12%). A frequency peak was observed during the warm season, between January and April. In collaboration with the Museo Argentino de Ciencias Naturales, the following species were found in the city of Buenos Aires during this period: *Eptesicus furinalis*, *L. blossevillii*, *L. cinereus*, *L. ega argentinus*, *Eumops bonariensis*, *Molossops temminckii*, *Molossus molossus* and *T. brasiliensis*. Since 1992 a total of 111 RABV strains, among which 109 were typed as variant 4 (98.2%), linked to *T. brasiliensis*, one was associated to *L. cinereus*, and one to *L. blossevillii*. A single spillover event was detected during this period, corresponding to a cat infected with variant 4, which bit three family members and the veterinary doctor.

EVALUATION OF THE OCCURRENCE AND POSITIVITY OF BATS SUBMITTED TO RABIES DIAGNOSIS IN THE STATE OF SÃO PAULO

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Over the years, the association of rabies surveillance with laboratory diagnosis permit to verify the importance of bats as reservoirs of the rabies virus. Due to the synanthropic habits and the ability to fly, which allows them to migrate and reach great distances, the control and surveillance of rabies in this species represents a major challenge. Therefore, the objective of this study was to evaluate the number of bats sent to the rabies diagnostic laboratory relating their origin in the state of São Paulo, with seasonal variations in the number of bats sent and the positivity for rabies. Hence, it was held data collection of bats received for the diagnosis of rabies in the period from 1996 to 2003, using Institute Pasteur's database. In the analyzed period, were sent 9932 bats, which 1380 (13,89%) were hematophage bats, 6005 (60,46%) non-hematophage e 2547 (25,645) were non-identified and from the total, 206 (2,07%) animals were positive for rabies. When evaluating the number of samples sent and positivity by Regional Epidemiological Surveillance Groups (ESG) of the State of São Paulo, we obtained the following results, described in number of bats sent/percentage of positive for rabies: Campinas 3177/2,55%; São João da Boa Vista 1000/2,9%; Ribeirão Preto 1574/0,89%; Presidente Prudente 19/52,63%; Taubaté 27/29,63%; Presidente Venceslau 33/24,24%. According to the results obtained it is possible to observe that positivity is not related to the number of bats sent by the municipalities, because the highest positivity was recorded in cities with fewer samples sent. This expressive variation of the positivity found in the analyzed period should be related to the surveillance strategy adopted in each municipality and the measures taken for containment. These results reiterate the relevance of epidemiological surveillance in bats and the need to evaluate the strategies adopted for the surveillance and control of rabies.

INFLUENCE OF SEASONAL VARIATIONS ON RABIES POSITIVITY IN BATS

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Bats are natural reservoirs of rabies, an acute and fatal encephalitis that affects the central nervous system of mammals. The influence of seasonality on reproduction, on genetic metabolism and on the activity pattern in bats have been studied, but little is known about the influence of climatic seasonality on the development of diseases. The objective of this work was to analyze the influence of seasonality on the positivity for rabies in the period from 1996 to 2003. A retrospective study was carried out, through the records in the reference laboratory database considering the total of animals sent, the positive diagnosis for rabies and the date of shipment. For the analysis of seasonality, the following months were considered: spring - September to November; summer - December to February; autumn - March to May; and winter - June to August. Positivity was calculated in relation to the seasons. To verify the existence of significant differences in the number of animals diagnosed positive between the seasons, the partial chi-square test was used for comparisons in pairs. A total of 9932 bat samples were sent to laboratory diagnosis and the positivity found in relation to the seasons were: spring 1.44% (51/3534); summer 1.63% (48/2946); autumn 3.18% (58/1821) and winter 3.00% (49/1631). Only in the comparison between autumn and winter there was no significant difference. The highest positivity was in autumn, followed by winter. The greater identification of positive animals in these climatic seasons can be due to drought in this period, causing the need to search for food and consequent change of shelter. Considering the analyzed period and the sampling of this study it can be concluded that there was a seasonal variation in the number of bats sent for diagnosis and positive for rabies.



ROUNDTABLES

ROUND TABLE 1

REGIONAL OPPORTUNITIES FOR THE ELIMINATION OF DOG AND CAT-MEDIATED HUMAN RABIES TRANSMISSION – (90 MIN).

Chaired by Mgr. Natalia Casas. Coordination of Zoonoses. National Direction of Epidemiology and Analysis of the Health Situation. Ministry of Health and Social Development, Argentina.

Co-chaired by Dr. Sergio Recuenco.

Introduction: The aim in the Americas is to eliminate human rabies transmitted by dogs, which is part of the Plan of Action for the Elimination of Neglected Infectious Diseases and Post-elimination Actions 2016-2022. Cases of human rabies transmitted by dogs decreased over the years, but cases are still reported in Bolivia, Haiti, and Guatemala. It is essential to achieve this aim, to carry out anti-rabies vaccination campaign in dogs and cats every year, with coverage of over 70%. Also, perform surveillance and monitoring of the people bitten to have the post-exposure prophylaxis in a timely and appropriate manner. As well, awareness on rabies and preventing dog bites are important. These activities should be developed in the endemic countries mentioned above, as well as in the countries surrounding them. The countries participating in the round table have experience in these actions and will share them. They are working strongly on eliminating human rabies transmitted by dogs.

Agenda and Speakers:

1. Eliminación de la rabia canina en América Latina. Estado de situación. Marco Antonio Natal Vigilato, DVM, PHS, MSc. PANAFTOSA-OPS/OMS (12 min).

2 Contribution to surveillance and management of canine rabies in the border Brazil-Bolivia: first descriptive and molecular analysis of the urban epizootic canine rabies, 2015. Dr. Juliana Arena-Galhardo, Epidemiology and Surveillance, Brazil.

3. Canine rabies epizootic study from February to July 2018 in Monte Cristo and San Antonio neighborhoods, Salvador Mazza, Salta, Argentina. Bioq. Carolina Campo, Epidemiology and Surveillance at Salvador Mazza Hospital, Public Ministry of Health, Salta.

4 Results of the vaccination campaign (2017-2018). Dr. Fleurinord Ludder, National Coordinator of Mass-Dog Rabies Vaccination Operations, Haitian Ministry of Agriculture – Animal Health Directorate, (MARNDR), Haiti.

CONTRIBUTION TO SURVEILLANCE AND MANAGEMENT OF CANINE RABIES IN THE BORDER BRAZIL-BOLIVIA: FIRST DESCRIPTIVE AND MOLECULAR ANALYSIS OF THE URBAN EPIZOOTIC CANINE RABIES, 2015

Galhardo, Juliana Arena; Neres-Gonçalves, Vitória Machado; Marques, Natalia Trindade Azevedo; Borges-Alcântara, Lilian Oliveira; Neves, Danielle Ahad; Ramos, Carlos Alberto do Nascimento

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Since 2006 the cross-border region Brazil-Bolivia is considered a warning area for dog transmitted rabies virus variant 1 (AgV1), previously unknown in Brazil. Until then, only canine rabies virus variant 2 was described circulating in Brazil. In 2015, a large canine rabies outbreak occurred in the region in which one person died after 30 years without human rabies cases in Mato Grosso do Sul State. Using the database and samples from the State Agency for Animal Health Protection, we held a descriptive and molecular analysis of canine rabies epizootic that occurred in Corumbá and Ladário Brazilian cities from January to December 2015. Canine and feline brain samples (N=388) were sent to laboratory rabies diagnosis by direct immunofluorescence and inoculation in mouse brain. There were 70 positive dog samples, 58 (82.9%) from Corumbá and 12 (17.1%) from Ladário. All feline samples were negative. Regarding the monthly surveillance record, the highest incidences were in April and July with a decreasing trend throughout the year. The human case was detected in April and patient deceased in May. There were no positive cases in November and only two positive samples in December. The general profile of canine samples submitted was predominantly young males up to 5 years old, mixed-breed and resident. There was no information about vaccination status. Five from the positive samples (5/70) were submitted to molecular analysis by RT-PCR and sequencing. These samples showed molecular homology of 99.3%-99.6% among each other and, compared to samples available in GenBank, were genetically related to samples from Peru, Bolivia and Argentina, suggesting a phylogeographical relationship. Failures in surveillance and public health management prior to 2015 contributed to epizootic and the virus variant was similar to other isolates from countries surrounding Bolivia, requiring international One Health Approach for mitigating canine rabies in Latin America.

ESTUDIO DE EPIZOTIA DE RABIA CANINA EN BARRIO MONTECRISTO Y SAN ANTONIO - SALVADOR MAZZA, SALTA, ARGENTINA ENTRE FEBRERO Y JULIO DE 2018

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¹Vigilancia Epidemiológica del Hospital de Salvador Mazza, MSP de Salta.

INTRODUCCIÓN. La rabia es una enfermedad viral zoonótica, infecta a mamíferos y causa encefalitis fatal. El día 8 de mayo de 2018 el Responsable de Zoonosis del Ministerio de Salud Pública notificó que un perro de Barrio Montecristo resultó positivo, donde la cobertura vacunal en mamíferos no superaba el 30%.

OBJETIVO. Conocer, fortalecer e intensificar la prevención, vigilancia y control de la rabia en B° Montecristo y San Antonio de Salvador Mazza desde febrero a julio de 2018.

METODOLOGÍA. Se implementó un estudio descriptivo retrospectivo. Se efectuaron tareas de vigilancia de canes por rastillaje en tres periodos (13 a 17/05, 29/05 a 01/06 y 28 a 29/06). Vacunación a fin de mejorar la cobertura con vacuna de cultivo en líneas celulares en el territorio comprendido entre las siguientes coordenadas: 22°04'05.79"S-63°41'52.93"O // 22°04'10.23"S-63°41'45.03"O // 22°04'19.12"S-63°41'53.48"O // 22°04'15.57"S-63°42'00.11"O desde febrero a julio de 2018.

Se realizó vacunación a humanos, aplicando esquema de Zagreb con vacuna de cultivo en líneas celulares a expuestos a accidentes no significativos y gammaglobulina antirrábica en posibles accidentes por lamedura de mucosas (en niños, adultos y ancianos).

Dos talleres educativos a vecinos en el barrio.

RESULTADOS. El caso mencionado inició sus síntomas el 2 de mayo. Se identificaron 6 perros que habían fallecido con síntomas compatibles desde el 2 de febrero hasta la fecha del positivo. Prospectivamente hasta el 14 de junio se detectaron en dicho territorio 10 perros sospechosos, de los cuales se extrajeron muestra para diagnóstico a 6, resultando 3 positivos por PCR. El agente identificado fue Virus rábico clásico (RAV) Variante I.

Recibieron esquema Zegreb 53 personas y 9 gammaglobulina específica (100%)

La cobertura canina ascendió a valores superiores al 90%.

Dos vecinos que concurren a los talleres vivenciaron episodios con sus canes lo que permitió la investigación.

CONCLUSIONES. Alcanzar la cobertura del 95% interrumpió la transmisión.

Fleurinord Ludder.

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Rabies is observed in Haiti from the colony (Lapole J. L. 1978). The first anti-rabies vaccination campaign was carried out in Haiti between 1926-1927 (Agricultural Bulletin, 1927). This control measure experienced a great expansion in terms of regularity and immunization coverage between 2006-2010. This regularity broke with January 12 earthquake. Since 2013, with the financial support of the World Bank, the Ministry of Agriculture has taken up the vaccination process. In 2017, with the above-mentioned support and technical support from Center for Disease (CDC), the ministry planned to vaccinate 780,000 dogs through a campaign focused on a new protocol: the mobile phone application to assign vaccination brigades, control vaccinators, determine geographical position of vaccinated dogs and vaccination coverage.

Then we have: i) used "STARC code", taking into account the population density and proximity of the community to a major road network to classify vaccination areas according to priorities (large, low, none), ii) defined areas averaging 400 dogs where there is priority, iii) assigned 2 vaccination brigades each equipped with 1 Smartphone connected to a server, to vaccinate dogs for 2-3 days in high priority areas and for 1 day in low-priority area using a mixing fixed-point, door-to-door and Capture-Vaccinate-Release techniques; iv) daily download of vaccination data to the server for accounting and analysis.

With this methodology, the ministry organized vaccination in 3 phases spread over a period of 430 days during which 306442 dogs were vaccinated in 52% of areas; This makes a total coverage rate of 43.3% against 73.3% in areas visited by the brigades. These covers were confirmed by a post-vaccination survey. After vaccination, the number of rabies cases confirmed by the laboratory decreased considerably.

This new protocol is very effective since it leads to the vaccination of 70% of vaccinated dogs in areas where it has been applied.

RABIES BIOLOGICS IN LATIN AMERICA

Chaired by Dr. Charles E. Rupprecht. LYSSA LLC.

Co-chaired by Dr. Gabriela Calamante. Institute of Biotechnology, CICVyA, INTA, Buenos Aires, Argentina.

Introduction: RABIES BIOLOGICS IN THE REGION - MILES TO GO BEFORE WE SLEEP?

Considering the thesis of the global elimination of human rabies by dogs (GEHRD) program and the progress in Latin America over the past 35 years, additional capacity (not less!) is needed now more than ever in the region. Basic purity, potency, safety and efficacy remain the foundation upon which rabies biologics have adhered in a post-Pasteurian world. Hence, as a modern benchmark, the concept of highly purified, serum-free rabies vaccines should become an aspiration for other industry adherents to emulate. By comparison, in the 21st century, there is no place for either Fermi or suckling mouse brain nerve tissue-based vaccines for humans or other animals. Similarly, gone are the days when the NIH test should be regarded as a measure of rabies vaccine potency, in light of current evidence-based approaches that focus upon evaluation of the viral glycoprotein trimers as a basis of the inducible immune response in pre-exposure or postexposure prophylaxis (PEP). Moreover, if safety is to remain paramount in the face of a growing anti-vaccine lobby (strengthened no doubt in part by recent unimaginable counterfeit rabies vaccines), then any post-marketing focus needs to extend into a routine vaccine adverse event reporting system, which has been lacking. Ideally, activity related to efficacy in the primary host should aim for the greatest breadth and longest duration of immunity possible, without the need for routine boosters, in line with most recent WHO/SAGE recommendations concerning route, dose and patient visitations. This is the challenge for new label claims. Clearly, while a focus upon vaccines is important, other biologics, such as the virus-neutralizing antibodies in RIG and MAbs, must not be ignored, especially as a part of actual PEP and a major focus upon thorough wound infiltration. Similarly, regional production of human and veterinary biologics are critical based upon targets and capacities, but are simply not enough. Human cases will continue to occur despite PEP without a concomitant concentration upon reservoirs and their respective viral pathogens. The rhetoric behind the GERHD will only become a cost-effective, long-term reality after the leadership shown throughout the Americas proceeds by actually eliminating canine rabies virus transmission, as well as the eventual initiation of oral rabies vaccination directed towards wild carnivores, due to apparent host shifts among these populations and purposely directed against the adaptive viruses which they perpetuate. In homage to LP and RF, our New World woods are lovely, quite dark and deep, but we still have several promises to keep.

Agenda and Speakers:

1. **Brief History about Rabies Vaccine Public Production in Argentina.** Dr. Oscar Perez. Viral Vaccines Laboratory, ANLIS - Malbran, Argentina.
2. **Rabies vaccine and anti-rabies serum produced at Brazil.** Neuza Frazati, Biol. Gerente de Projetos Vacinas Virais. Secretaria de Estado Da Saúde, Brazil.
3. **A possible way to stimulate the development of new vaccines in Latin America.** Dr. Alejandro Parola. Pablo Cassara Foundation, Argentina.
4. **Rhabdo-like recombinant Viral Like Particles (VLPs): a new generation veterinary rabies vaccine. From the idea toward the market.** Dr. Diego Fontana, Centro Biotecnológico del Litoral – Facultad de Bioquímica y Ciencias Biológicas - UNIVERSIDAD NACIONAL DEL LITORAL.
5. **Adventitious Virus Control In Production of Rabies Vaccine.** Dra. Irene Álvarez. INTA, Argentina.

BRIEF HISTORY ABOUT RABIES VACCINE PUBLIC PRODUCTION IN ARGENTINA

Dr. Oscar Pérez.

Malbrán Institute, Argentina

During 1885 Dr. Pasteur developed the first treatment for rabies transmitted by dogs, in humans. This fact was the reason why Dr. Davel was sent to the Paris Pasteur Institute to be capacitated at the methodology. He came back to Argentina with a rabies virus strain to begin the vaccine production. The first human treatment with rabies vaccine in Latin America was on the 3th of September of 1886.

Dr. Carlos G. Malbrán was a doctor and microbiologist and he had a great interest in public health policies, so he was elected National Senator. He proposed the law to create the Bacteriology Institute that was finally inaugurated in 1916. In 1940 was named as the National Microbiology Institute.

At the same time were implemented health public policies from the National State and, in 1918, in Cordoba city, the university students, promoted the University Reform. This fact impacted on the superior education policy till the present.

The Buenos Aires Bacteriology Institute had the aim to make the rabies vaccines production. The first one was the Semple Vaccine, then the Fermi Vaccine and the last one the Fuenzalida –Palacios Vaccine, this one was used to stop the 1976 outbreak that had a major impact in Buenos Aires city.

Since 2009 the Health Ministry replaces the use of first generation human vaccines for the imported second generation ones. The production of rabies vaccine at A.N.L.I.S (ex Malbrán Institute), was discontinued without established a technology replacement. Then, the Rabies Vaccine Production Laboratory tend to adopt strategies to develop second and third generation vaccines.

At the presentation in the RITA Conference are being explained the historic facts that have had impact at the sanitary policies and how the new developments were implemented, the results and the actual viability of rabies vaccines public production.

“RABIES VACCINE AND ANTI-RABIES SERUM PRODUCED AT BRAZIL”

Neuza M Frazatti-Gallina, Claudia R M Botelho and Regina Mourão-Fuches
Instituto Butantan

Four Brazil public institutions manufacture vaccines and anti-rabies serum (from horses): Butantan Institute (SP), Vital Brazil Institute (RJ) Ezekiel Dias Foundation (MG) and Institute of Technology of Paraná-TECPAR (PR). The first institute produces rabies vaccine and anti-rabies serum for human use, the second and third, produce only anti-rabies serum. The TECPAR manufactures vaccine for cat and dogs. Butantan Institute has started the rabies vaccine production in 1957 with the Semple type. However, in 1964, this technology was replaced by Fuenzalida & Palacios rabies vaccine and after to Fuenzalida & Palacios modified vaccine (1987). Since 2002, the Brazil Ministry of Health uses, for human immunization, only Vero cell or duck embryo rabies vaccines. A new rabies vaccine with Vero cells and solid microcarriers cultivated in serum free medium in bioreactor was developed at Butantan in 2000.

The clinical trials of this rabies vaccine finished in 2004 and the product was registered at ANVISA in 2008. After that, the manufacture process was scale up to bioreactor of 30L and 150L. A factory, with manufacturing capacity of 8 million doses of vaccine per year, was built for the new rabies vaccine production. Currently, the biggest challenge for countries of Latin American that manufacture rabies vaccine and/or anti-rabies serum is the adaptation of the currently manufacturing process to the new technologies and GMP (Good Manufacturing Procedures). This change requires high costs, with investments in buildings, utilities, operational training and mainly an efficient quality assurance department. The Butantan has invested in the GMP and in 2017 started a new serum factory.

A POSSIBLE WAY TO STIMULATE THE DEVELOPMENT OF NEW VACCINES IN LATIN AMERICA

Parola A.D.^{1,2}

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² Instituto de Ciencia y Tecnología Dr. César Milstein – CONICET. Saladillo 2468, Buenos Aires, Argentina

The development of rabies vaccines in Latin America (LATAM), specifically in Argentina, began early in 1886 with the first application by Dr. Davel. Since this time LATAM Countries stimulated Public Institutions as vaccines producers. Health public policies plus vaccines had significant impacts on public health in the region. For different reasons during the 80s and 90s, the public vaccine production facilities were deactivated, except for a few exceptions. Since the 90s the Argentine government has stimulated the production of biotechnological and biological drugs. In 2008, Pablo Cassara Laboratory (PCL) got public fiscal funds for the development of a chromatographically purified cell culture-derived human rabies vaccine. Within this framework, the Pablo Cassara Foundation joined the project providing an ad hoc group of professionals with experience in Virology from the Public Universities. This group focused on the development of cell banks, cell culture, and viral purification among others, additionally, the PCL contributed with more than 40 people from its different specific working areas in order to elaborate different pilot lots. A regulatory dossier was submitted in January 2018 and is in evaluation by the national authorities. We hope soon to be able to advance with the clinical trials to be able to begin the commercialization of this vaccine. This achievement would not have been possible without the constant support of the Pablo Cassará Laboratory during the last ten years. The National Administration of Drugs and Food (ANMAT) who put this project under the Innovation Support plan also helped to boost the project. On the other hand, many alliances were established with Public Control, Research and Development laboratories like ANLIS Malbrán Institute, National Institute of Agricultural Technology (INTA), National University of La Plata, among many others.

In brief, the existence of companies with focus in innovation that able to support economically in a sustained manner over time, and the Government support, demonstrated with fiscal measures or collaborations in the area of regulation added to an ecosystem of Public Institutes capable of providing complex services, are essential elements to promote the development of vaccines in LATAM.

RHABDO-LIKE RECOMBINANTE (VLPs): A NEW GENERATION VETERINARY RABIES VACCINE. FROM THE IDEA TOWARD THE MARKET

Diego Fontana¹, Federico Marsili¹, Marina Etcheverrigaray¹, Ricardo Kratje¹ y Claudio Prieto^{1,2}

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Virus-like particles (VLPs) are self-assembled macromolecular structures that mimic the native conformation of a virus. As they are highly immunogenic, absolutely non-infectious and non replicating particles they are excellent vaccine candidates.

In this work we present the development of a rabies virus-like particle (RV-VLPs) based on the expression of the rabies glycoprotein in HEK293 cells. RV-VLPs were fully physicochemical and biochemically characterized and the immune response induced in experimental animals was analysed, confirming the induction of high neutralising antibodies titers. Further, the protective capacity of RV-VLPs was assessed by the NIH potency test for rabies vaccine, even without adjuvant addition in the vaccine formulation.

Later, the bioprocess development was achieved adapting the obtained producer HEK293 clone from adherent conditions to suspension, using serum free medium. The production of RV-VLPs was scaled-up from erlenmeyer to 1 L and 5 L bioreactor operated in continuous mode with perfusion, maintaining the productivity and product quality. Due to this clone is able to produce continuously the VLPs to the supernatant without losing productivity, the culture can be maintained in time with high viabilities in order to obtain the vaccine doses needed. This feature represent an advantage in comparison with the classical rabies vaccine production processes where some days after infection the total harvest of the culture has to be performed and the process re-started again. With the RV-VLPs technology we are able to produce up to 85,000 doses in 20 days with a 5 L bench top bioreactor.

Finally, the innocuity and immunogenicity of RV-VLPs was confirmed in dogs, demonstrating that this new generation vaccine candidate is able to induce high titers of neutralizing antibodies without any observed adverse effect.

Thus, this results show that RV-VLPs, that will be presented commercially as Rhabdo-like recombinante (VLPs), represent an excellent option to be incorporated into the market as a new generation, recombinant and virus free rabies vaccine.

ADVENTICIOUS VIRUS CONTROL IN PRODUCTION OF RABIES VACCINE

Alvarez Irene^{1,2}, Franco Lautaro¹, Perez Lopez Jorgelina¹, Politzki Romina¹, Ruiz Vanesa², Schammas Juan Manuel¹, Trono Karina^{1,2}.

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Viral vaccines, viral master seeds, and cell lines used to produce them must be tested to ensure that they are free of contamination with adventitious agents, such as viruses. There are three main complementary approaches used to control the potential adventitious viral contamination of biologicals products: (1) Selecting the source material, (2) Testing of source materials and products originated during the manufacturing process for the absence of detectable virus though, (3) Testing the capacity of the manufacturing process to remove or inactivate viruses.

The standard adventitious control scheme of Rabies vaccine should include the inoculation of a sample into many susceptible indicator cell lines capable of detecting a wide range of human and relevant animal viruses, and also should be inoculated into laboratory animals (suckling and adult mice), and into embryonated eggs to reveal viruses that cannot grow in cell cultures. The specific detection of the viruses should be performed by immunoassays.

The Adventitious laboratory unit at Virology Institute of INTA has the facilities approved by Argentina's National Sanitary Authority in animal health (SENASA) required to perform the adventitious contaminant control in biological products. Every study is designed in accordance with the national and international regulatory guidelines.

DIAGNOSTICS STATUS QUO

Chaired by Dr. Subbian Satheshkumar Panayampalli, Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology National Center for Emerging and Zoonotic Infectious Diseases Centers for Disease Control and Prevention USA. Introduction (5 min).

Co-chaired by Lic. Susana Russo. National Reference Laboratory, National Service for Agri-Food Health and Quality (SENASA), Rabies Department. Summary (5 min).

Introduction: Rabies, a 100% fatal disease (after symptoms onset) is preventable by administration of appropriate post exposure prophylaxis immediately after exposure. Hence, expedited and accurate diagnosis for presence of rabies virus infection in animals is of high importance. However, there are challenges with diagnostics, (1) availability of reagents for newer diagnostic methods recently approved by World Organization of Animals Health, (2) validation of reagents against RABV variants prevalent in a particular region, (3) ability to perform human ante-mortem diagnosis involving multiple samples, (4) lack of anti-virals and treatment for human rabies, and (5) surveillance data to determine the burden of rabies, with particular emphasis on bats. In addition: competency, proficiency, accreditation and quality assurance of laboratories conducting rabies diagnostics are important factors that needs attention. This roundtable features five presentations that will address various aspects of these issues under the title of existing conditions in different countries and pathway for future improvements.

Agenda and Speakers:

1. **The Point of View of the National Program in the Accreditation and Certification Experience for the Rabies Laboratory Diagnostics in Mexico.** Dr. Verónica Gutiérrez Cedillo, Ministry of Health, Mexico.
2. **National Network of Public Health Laboratories for the diagnosis of rabies in Mexico.** Dr. Nidia Aréchiga Ceballos. Rabies Laboratory, Department of Virology, Institute of Reference for Diagnosis and Epidemiology, Mexico.
3. **Diagnosis and identification of rabies virus variants in Chile.** Dr. Veronica Yung. Public Health Institute, Rabies Section Chile.
4. **Rabies diagnostics in Brazil.** Carla I. Macedo, Vet. MSc. Pasteur Institute, São Paulo, Brasil.
5. **A review of updates to the World Health Organization and the World Health Organization for Animal Health recommended rabies diagnostic methods.** Dr. Susan Moore. Kansas State University, College of Veterinary Medicine, Veterinary Diagnostic Laboratory, Manhattan, KS.

THE POINT OF VIEW OF THE NATIONAL PROGRAM IN THE ACCREDITATION AND CERTIFICATION EXPERIENCE FOR THE RABIES LABORATORY DIAGNOSTICS IN MEXICO

Verónica Gutiérrez Cedillo; Fernando Vargas Pino.

Ministry of Health/Mexico

The process of the elimination of the dog bites rabies transmission in Mexico is one of several proposals from the Priority Transmissible Diseases Programs of Prevention and Control of the Mexican Ministry of Health. On them is clearly recognized the risk and importance for the community to live together with rabies, and the importance of its elimination.

To achieve this goal it was necessary to spend several years of permanent work from Federal Government coordinated by the Ministry of Health, and the formally started in the last quarter of the past century but showed the significant advance during the first decade of this century.

The compliance of this proposal is based in a sensitive system of surveillance and register with the opportunity to evaluate the incidence and the disease impact. It is based in the quality and opportunity of the rabies diagnostic efficacy. For the human cases and suspicious animal rabies cases it is essential the rabies virus characterization of virus isolated individually or in outbreaks to identify the original host associated with its geographic reference.

It is not possible without the governmental and political decision to continue running the activities to eliminate this disease in the national territory as was described in the Six Year Period National Programs of the Federal Government since the 70's years, where was officially recognized the importance for the public health the rabies control in Mexico. The same interest was ratified designating the budget and priority in the past years until 2005, where no more human rabies cases transmitted by dogs were reported, but also in 2015, were reported the last dog rabies case with the dog rabies V1 variant.

The State Public Health Services established an efficient system to answer to the rabies control needs according with the National Objectives and Strategies, but with a new relationship among the Federal and States Authorities to define the proper goals and the achievement of the objectives that are currently on place.

NATIONAL NETWORK OF PUBLIC HEALTH LABORATORIES FOR THE DIAGNOSIS OF RABIES IN MEXICO

Rabies Laboratory at InDRE

Nidia Aréchiga Ceballos.

Laboratorio de Rabia, Departamento de Virología, Instituto de Diagnóstico y Referencia Epidemiológicos, Mexico

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In Mexico, the General Direction of Epidemiology (DGE) is in charge of the epidemiological surveillance of Rabies, while laboratory diagnosis allows to strengthen epidemiological surveillance and monitoring of the rabies virus. The implementation of strategies for the prevention and control of rabies are established jointly with the National Zoonoses Program of the National Center for Preventive Programs and Disease Control (CENAPRECE). The National Network of Public Health Laboratories (RNLS) is the set of laboratories with specific objectives that allow the unification of diagnostic methods, criteria for interpretation of results, technology transfer, knowledge generation and human resources training that guarantee technical-administrative procedures that produce useful laboratory information for epidemiological surveillance and the operation of preventive programs. It is the technical-scientific support useful for epidemiological surveillance, generates quality information for timely decision making, through confirmation by laboratory studies in biological samples. The Rabies Laboratory Network it is structured in three levels: national, state and local or their equivalents for other institutions. The national level is represented by InDRE as a National Reference Laboratory (NRL). In the National Network of Public Health Laboratories (24 states) they have implemented the diagnosis of Rabies using the direct immunofluorescence test. These Laboratories have undergone quality control until the diagnosis has been released and last year (2017) in situ supervisions were carried out to each one of the laboratories with the objective of granting them the technical competence to those that meet the requirements established in the ISO: 9001: 2015 and ISO: 15189: 2012 standards.

Veronica Yung P., Jaime Lagos B., Michelle Lineros S., Jorge Fernández O.

Instituto de Salud Pública de Chile. Sección Rabia. Subdepartamento de Genética Molecular.

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To prevent rabies is required rapid and accurate laboratory diagnosis in animals and timely administration of post-exposure prophylaxis. Traditionally, rabies has been diagnosed using direct fluorescent antibody (DFA) test to detect rabies virus antigens in brain tissue and at least one of the following confirmatory tests: mouse inoculation test (MIT), rabies tissue culture infection test (RTCIT) and reverse transcription polymerase chain reaction (RT-PCR), although exist other test with great application potential, laboratory tests could be standardized, sensitive and specific. For human rabies diagnostics ante-mortem no single test is sufficient, several tests are necessary. The use and the criteria for selecting diagnostic assays will vary according to the sample analyzed or depending on whether the setting in endemic or non-endemic.

In Chile, dog rabies has been controlled and insectivorous bats have been identified as the main rabies reservoir, since then the circulating variants have been determined to define current epidemiologic patterns of disease and provide appropriate information for the development of rabies control programs. In the country 14 bat species have been identified, which are shared with bordering countries, over the last years, 4 insectivorous bats species are established as reservoir of rabies virus with sporadic transmission to domestic animals.

Recently three rabies virus isolates belonging to *Lasiurus* and *Myotis chiloensis* bats were analyzing by nucleoprotein sequences and comparing with database of other bat species of America, indicate that this isolates present a 94 % similarity with variants corresponding to *Eptesicus* bat, even though this species has not been identified in Chile, it circulates in southern Argentina, this finding could indicate the presence of a new variant in Chile and suggests a spillover, considering the migratory habits of insectivorous bats.

Therefore, the surveillance is important on different bat species and the research with molecular tools to understand the biological complexity between viral variants and their reservoirs in the Americas region.

Carla Isabel Macedo

Instituto Pasteur de São Paulo, Brasil

In the last 20 years, the laboratory rabies diagnosis has undergone major evolution, starting with the implantation of modern virological techniques, antigenic and molecular studies, and association with epidemiological investigation. The use of cell culture for isolation of rabies virus was essential to reduce the cost of diagnosis and dramatically the number of animals used. Fluorescent antibody test should be standardized for the laboratories of the same region with the use of the same antinucleocapsid conjugate. Antigenic and genetic characterization should also be standardized, with the same panel of monoclonal antibodies, and the sequencing of a same fragment of viral RNA, respectively. The panel reaction profiles should define the main variants of the certain region, currently identified. New variants, such as those isolated from some genera of insectivorous bats (*Histiotus* spp, *Nyctinomops* spp, *Myotis* spp), were frequently determined with reproducibility of the antigen. New variants of the terrestrial cycle have also been identified in the last 20 years. The production of a new panel, or even the re-use of the panel defined by the CDC, become essential for the prompt determination of the source of infection. Each case of human rabies should have its source of infection rapidly determined, to conduct epidemiological research and control actions. Evidently the study of each case of human, canine and feline rabies should be complemented with genetic sequencing. Neither should we ignore the importance of morphometric and / or genetic identification of wild species, which act as reservoirs. The deficiency of human, material and technological resources in most of the countries of the Americas must be highlighted, given the numerous zoonosis that occur on the continent. The risk of underreporting of rabies cases becomes very high if there is no full development and dedication of the laboratory network and epidemiological research in real time.

A REVIEW OF UPDATES TO THE WORLD HEALTH ORGANIZATION AND THE WORLD HEALTH ORGANIZATION FOR ANIMAL HEALTH RECOMMENDED RABIES DIAGNOSTIC METHODS

Susan M. Moore, PhD, MS, HCLD (ABB), MT (ASCP) SBB

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In 2018, the World Health Organisation for Animals Health (OIE) Terrestrial Manual, Rabies Chapter was updated and the World Health Organization (WHO) Expert Consultation on Rabies Third report was published. Both documents serve as guidance for rabies prevention and control measures including rabies diagnostics. This review will present some changes in recommendations and description of newer methods. Included are mention of methods to transport samples on filter paper, advantages and disadvantages of ELISA kits for antibody surveillance, and use of the direct rapid immunohistochemistry test (DRIT) for primary diagnosis. Reliability of results depend on both the sample selection, collection and transport and quality assurance of the method.

In particular, meaningful results are assured by selection of the appropriate test as 'fit for purpose' and proper verification/validation of the method. In areas of poor resourcing, difficulties in obtaining biological reagents necessary could be eased by development of a 'biological bank' and a WHO or OIE list of pre-approved kits with clear guidance for use. One challenge for guidelines is a need to develop directives regarding the hierarchy of tests based on the evolution of surveillance in a region.

ROUND TABLE 4

BAT RABIES

Chaired by Dr. Luis Lecuona. USDA, APHIS, International Services, Wildlife Program, Mexico City, Mexico.

Co-chaired by Méd. Vet Fernando Beltrán. Instituto de Zoonosis Luis Pasteur, Buenos Aires City, Argentina.

Introduction: Rabies in bats is currently a problem in many regions in the world. Rabies transmitted by haematophagous and not haematophagous bats is a problem that generates risk of transmission for domestic and wildlife animals, and more importantly for the impact that it represents in public health from many countries. In the Americas the common vampire or *Desmodus rotundus* is the most important but not the unique reservoir of the rabies virus variants detected in bats. During this session we will be posted about experiences in the Latin America transmission. We hope it will be useful for all of you and helps to develop efforts for intervention alternatives to reduce rabies in these animals and collaborate with efforts for efficient plans to manage the risk of rabies in bats.

Agenda and Speakers:

- 1. Challenges for prevention and control of human rabies transmitted by hematophagous bats in the Amazon Basin.** Dr. Sergio Recuenco. Center for Technological, Biomedical and Environmental Research (CITBM) / UNMSM; National University of San Marcos, Lima Peru.
- 2. Outbreak of human rabies in the Brazilian Amazon.** Dr. Livia Casseb. Evandro Chagas Institute, Brasil.
- 3. An overview of bat rabies in Argentina.** Méd. Vet. Fernando Beltrán. Instituto de Zoonosis Luis Pasteur, Buenos Aires, Argentina.
- 4. Rabia Paresiante en Argentina, aumento del área endémica. Medidas sanitarias.** Méd. Vet. Gabriel Russo. National Service for Agri-Food Health and Quality (SENASA), Argentina.
- 5. Bat Rabies in the United States.** PhD Microbiologist James Ellison. National Center for Emerging and Zoonotic Infectious Diseases, CDC, USA.
- 6. The continual discovery of novel lyssaviruses across the Old World.** Dr Ashley C. Banyard (PhD), Wildlife Zoonoses and Vector Borne Diseases, Animal and Plant Health Agency, New Haw, Addlestone, Surrey, UK.

CHALLENGES FOR PREVENTION AND CONTROL OF HUMAN RABIES TRANSMITTED BY HEMATOPHAGOUS BATS IN THE AMAZON BASIN

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Significant progress on canine rabies elimination in Latin America lead to the elimination of human rabies cases due to the terrestrial cycle in most countries. By the other side, human rabies outbreaks transmitted by wildlife, mainly due to hematophagous bat bites, are regular occurrence in the Amazon Basin, and sporadically in Central America. While canine vaccination is available to control rabies in dogs, this strategy is not available for bats or other wildlife in the region. The standard strategy to prevent new human cases is the administration of post-exposure prophylaxis (PEP) to people bitten. Though effective, access to PEP have is serious challenge in the Amazon Basin due to the disperse and remote location of populations at risk. Additionally, the high frequency of bat bites to people and the lack of recognition of the risk implied by those bites results in high risk for rabies for a vast region in the South American continent. Ideally, preventing new cases of human rabies due to the sylvatic cycle should be based on avoiding bites. Unfortunately, all strategies proposed for stop bites had been ineffective of its application was insufficient or impossible in the region.

Intervention with massive pre-exposure prophylaxis (Pre-EP) for populations at high risk had shown an immediate impact in Peru, stopping human rabies outbreaks in the geographical area intervened. Despite the success of massive Pre-EP, the logistics to outreach the populations in the Amazon Basin and achieve the desired coverage is still a major challenge, with large areas at high risk pending for the intervention. Additional challenges, such as sustainability of the massive vaccination program, ongoing efforts to avoid bites, introduction of more efficient vaccination schedules with intradermal vaccination, availability of vaccine, and inclusion of rabies vaccine in the child vaccination schedule, deserve discussion and a position by each Amazon Basin country. In the meantime, without other ways to effectively stop bites to people living in the Amazon Basin, massive Pre-EP for high risk population, is the strategy of choice to prevent human rabies in the region. Implementing massive Pre-EP program, according with risk criteria is urgent for the region.

OUTBREAK OF HUMAN RABIES IN THE BRAZILIAN AMAZON

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Until the mid-1990s, the state of Pará, the eastern portion of the Amazon, had the third highest rate of rabies cases in Brazil. According to the Brazilian Ministry of Health, only nine cases of rabies in humans (transmitted by hematophagous bats) were recorded in Pará between 1999 and 2002, all in the southeastern portion of the state. On the other hand, from 2003 to 2004, the number of registered cases more than doubled to 22 and was more generalized, ranging from Southeast to Northeast.

Between 2006 and 2017 cases of animal rabies occurred in several regions of the state of Pará, but no outbreaks were recorded in humans, in the Marajó region there were no human or animal cases recorded, but in 2018, in the municipality of Melgaço (50° 43' 1"W, 1° 48' 17"S), located in the region of Marajó Island, state of Pará, 250 km from Belém, the state capital; whose access to Belém is made by the Amazon River. Municipality with 24,789 inhabitants (3,66 inhabitants / km²). In which the main economic activities are the logging, agriculture (especially the cultivation of cassava) and fishing with hook and line; Cattle grazing is unusual. Eleven cases were recorded in people belonging to communities around the Laguna river, with 7 laboratory confirmed cases and 4 initial deaths (epidemiological link). National treatment protocol was applied in 6 patients, but without success. All isolates were characterized antigenically and genetically as variant 3 of *Desmodus rotundus* (AgV3).

Although rabies control measures have improved in many South American countries, transmission of the disease by bats has increased and has become a public health concern, and several human cases have been detected. Outbreaks of rabies transmitted by bats have occurred in several remote areas in Peru, Venezuela, and Brazil. Finally, public health campaigns should be conducted to alert residents of remote small communities in the Amazon to the risk of bite bites in the transmission of rabies and the need of all persons reporting bats attacks undergoing post-exposure treatment to prevent other cases of rabies. rage transmitted by vampire bat. In addition, ecological studies should be initiated to clarify the dynamics of rabies infection among populations of *D. rotundus* in affected areas.

AN OVERVIEW OF BAT RABIES IN ARGENTINA

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The first bat positive to rabies virus (RABV) at the Instituto de Zoonosis Luis Pasteur (IZLP) was recorded in 1965. The bat was found in the port of Buenos Aires (CABA) and belonged to the species *Lasiurus ega argentinus*. After 26 years without positive cases, in 1991 a specimen of *Tadarida brasiliensis* was captured in the neighborhood of Barracas and diagnosed positive for RABV. The bat had bitten a girl which was successfully treated at Hospital "Carlos G. Durand". Since that episode, an increasing number of cases in different bat species frequently associated with contact or bites in pets and/or humans were observed. In 1992 monoclonal antibodies (CDC) antigenic characterization techniques were incorporated, while molecular diagnosis and typing had been implemented since 2010.

The IZLP belongs to the RABV diagnosis laboratories network of Argentina and regularly receives samples for diagnosis and isolations for variant genetic determinations from laboratories which are members of the national network. In the period 2007-2018, from a total of 4348 samples submitted to the IZLP, 328 (7.5%) cases were diagnosed positive, of which 284 (86%) were typed. Some of the positive samples represent spillover events. The most prevalent variant was V4 (59.71%), associated with *Tadarida brasiliensis*, which was also found in 5 dogs, 4 cats, and a red fox. Variants 3 and 3a are associated with *Desmodus rotundus* (23.31%). A RABV V3 was isolated from a crab-eating fox. Other lesser prevalent variants were isolated: *Myotis* spp (2.82%) including a dog, *Eptesicus* spp (1.41%) with 2 cases in cats, *Histiotus* spp (1.41%), *Lasiurus* spp (1.76%). The remaining correspond to terrestrial variants V1 (8.48%) and V2 (1%).

Spillovers had been registered mostly during temperate to warm months in the provinces of Córdoba, Buenos Aires, and the remaining in a minor proportion were found in CABA, Chaco, Salta, and Río Negro.

BAT RABIES IN THE UNITED STATES

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The connection between bats and rabies is one of the oldest and most thoroughly researched bat-associated zoonotic disease. In North America, the greatest diversity of rabies viruses is found within the Order Chiroptera and new rabies viruses are being described regularly. Over the last 20 years, the majority of human rabies cases in the US were identified as variants typically detected in bats. In the US, monitoring passive RABV surveillance trends enables public health authorities to perform more accurate risk assessments. Differences in temporal and spatial trends in the detection of RABV by bat species indicate the importance of collecting and reporting taxonomic data. In 2015, for the first time since public health surveillance for rabies began in 1944, bats were the most frequently reported rabid animal in the US, surpassing raccoons. Numerous factors could account for these observed trends, including changes in the overall view of surveillance, modifications in sample submission policies, alterations in public perceptions of the risk of rabies in bats, and population fluctuations. CDC has recently investigated numerous mass bat exposure events which pose a significant and unique public health concern, not only because of the high fatality of rabies, but also because of the potential for numerous human exposures that must be investigated, assessed and appropriately treated. This presentation will characterize the current epidemiological situation of bat rabies in the US, and discuss novel prevention and control measures for disease management in this unique reservoir.

BAT RABIES: THE CONTINUAL DISCOVERY OF NOVEL LYSSAVIRUSES ACROSS THE OLD WORLD

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Rabies is one of the most ancient diseases known to man, being first described in the 4th century BC. Classical rabies virus (RABV) is the archetypal virus classified within the lyssavirus genus, a group of diverse viral pathogens that are capable of causing the invariably fatal encephalitis, termed rabies. RABV is associated with the infection of both terrestrial carnivores and bats and remains an important but neglected viral zoonosis that is estimated to cause over 59,000 human deaths annually. Alongside RABV, novel lyssaviruses continue to be reported globally. Non-rabies lyssaviruses were first described in the 1950s in Africa and Europe where bat infections were reported.

Characterisation of these viruses was initially performed serologically and an early understanding of the lack of serological cross-reactivity from rabies vaccines was developed. Since then the genetic characterisation of these viruses has resulted in 16 distinct lyssavirus species being described with a further two viruses awaiting classification within the genus. The true human burden of these non-rabies lyssaviruses remains unclear, although a handful of human fatalities have been reported.

The advent of genetic characterisation of viruses and an increased interest in bats as reservoirs of viral diseases has meant that novel lyssaviruses continue to be reported. Epidemiologically, whilst classical rabies is present in terrestrial carnivores across the globe, the infection of bats with classical rabies appears restricted to the Americas. In contrast, all of the other lyssavirus species described have been detected in the Old World, predominantly in bats but occasionally in terrestrial carnivores. Here we describe the current diversity across the lyssavirus genus and whether they are of relevance to future rabies control strategies.

WILDLIFE RABIES

Chaired by Dr. Ivanete Kotait. Venco Saúde Animal - Londrina, Parana, Brasil.

Co-chaired by Dr. Daniel Cisterna. Instituto Nacional de Enfermedades Infecciosas (INEI), ANLIS "Dr. Carlos G. Malbrán". Ciudad Autónoma de Buenos Aires, Argentina.

WILDLIFE IN LATIN AMERICA

Ivanete Kotait – VENCO Saúde Animal S.A.

In discussion about wildlife rabies we compare the field structure with North America countries. In Latin America (South America and Caribbean) differently of United States, Canada and Mexico have not an epidemiological surveillance programs to know the occurrence of rabies, because the Government Departments from Agriculture and Environment are not responsible to zoonosis in wildlife. Passive surveillance based on clinical suspicion and syndromic surveillance may account for the majority of cases that are underestimated. It has been recognized that there has been limited systematic evaluation of rabies wildlife surveillance carried out within the region. The main reservoirs in South America are *Urocyon cinereoargenteus* (Colombia), *Cerdocyon thous* (Brazil), *Pseudolapex* spp (Brazil), *Callithrix jacchus* (Brazil), *Desmodus rotundus* (all countries) and insectivorous bat like *Tadarida brasiliensis* (Chile, Brazil and Argentina) and *Lasiurus* spp, *Molossus* spp (Argentina, Brazil). Rabies human cases occurred in Brazil, Equador, Peru and Colombia transmitted by vampire bat; transmitted by *Callithrix jacchus* in Brazil; transmitted by *Cerdocyon thous* and *Urocyon cinereoargenteus*, respectively in Brazil and Colombia. In Caribbean, the mongoose and vampire bat are the most important reservoirs with human cases in Cuba, Grenada, Porto Rico and in Grenada, French Guyana, Guyana, Suriname and Trinidad, respectively. Antigenic and genetic variants of rabies (AgV) can be identified by monoclonal antibodies techniques and genetic studies. Even the control of canine rabies is a true in South America, and distant reality in Caribbean countries, the approach of wildlife rabies control is very difficult at present, because it is an unknown challenge.

Agenda and Speakers:

1. **Wildlife rabies in Brazil.** Silene Manrique Rocha, MV. MSc Saude Animal. Federal University of Brasilia, Brazil.
1. **Persistence of wild terrestrial rabies in northeastern Argentina.** Dr. Daniel Cisterna. National Institute of Infectious Diseases, ANLIS Malbran, Buenos Aires, Argentina.
3. **Tackling Wildlife Rabies in North America: Fighting the Good Fight with Hard and Soft Science.** Richard B. Chipman, MS, MBA, USDA, APHIS.
4. **Oral Vaccination against Rabies; possibilities and hurdles beyond the obvious terrestrial reservoir species.** Adriaan Vos PhD. IDT Biologika GmbH, GERMANY

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In Brazil, although rates of human rabies have declined because decreases in rabies among dogs and cats, a concerning trend is the increasing rate of rabies among wildlife (wild canines, marmosets, bats non-hematophagous and hematophagous). Urban sprawl has brought wildlife in greater proximity to humans, bringing a concern about the reintroduction of rabies in urban environments from wild-cycle reservoirs. This descriptive study shows data registered in the Ministry of Health of Brazil, from 2002 to 2017 out using records of rabies infection according to number of cases, place and year of infection, to either human or animals. In the study period, 142 human cases were reported; the incidence rate was of 0.06/100,000 habitant. The proportion per cycle of transmission was 35.3% and 64.7% to urban and wild, respectively. Cases of human rabies transmitted by wild animals predominantly occurred in rural areas of North and Northeast regions, and 86% of those did not receive anti-rabies prophylaxis. Among the 3,554 wild mammals confirmed for rabies within the study period, bats were the most frequent vectors of the disease, representing 81.1% of all notifications. Infected bats were predominantly located in the Southeastern region. Other infected mammals (479 wild canids, 69 marmosets and 08 crab-eating raccoon) were mostly located in Northeast region. Center-West and North regions had a lower proportion of rabies among wild mammals, but of infected cattle were noticed, evidencing the role of the hematophagous bat as a maintainer of rabies virus in rural cycle. In 86 infected domestic animals (42 dogs and 29 cats) were found antigen varieties originated from wild mammals. Of those 42 infected dogs, 28.0% of cases were transmitted by bats and 72.0% by wild canids. The present results are useful for planning the control of wild rabies in Brazil.

Keywords: Wild rabies, Epidemiology, Spatial surveillance.

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In the Northeast of Argentina (NEA), rabies continues to be detected in dogs and different species of wild terrestrial animals. The majority of cases occurred in a geographic area focused on Formosa and Chaco provinces. In this work, we present the preliminary results of the phylogenetic analysis of rabies isolates obtained from fox (*Cerdocyon thous*), aguara guazú (*Chrysocyon brachyurus*), coati (*Nasua nasua*) and aguará pope (*Procyon cancrivorus*) collected between 2001 and 2013 in these provinces. In addition, historical samples from Argentina (1996), Brazil (2005) and Paraguay (1989-1991) and current samples of Paraguay (2015-2016) were included for comparison.

Phylogenetic analysis shows that the Argentine strains were divided into two large groups. In the first group, current samples from Chaco and Formosa (2001-2009) were clustered into two lineages according to geographic source. Each lineage contained both samples of dogs and wild animals. In addition, this group included two recent samples from Paraguay (2015-2016). On the other hand, the historical samples of the second group, segregated into two monophyletic lineages, with strains from Tucumán and Jujuy (1996) and Paraguay (1996). Finally, the strains of Brazil were distributed in a third group that included two lineages supported by dogs and foxes, respectively. These data show the complex epidemiology of rabies among the different wild species of Argentina and Paraguay. It is necessary to obtain more information implement an efficient control of rabies in that region.

TACKLING WILDLIFE RABIES IN NORTH AMERICA: FIGHTING THE GOOD FIGHT WITH HARD AND SOFT SCIENCE

Richard B. Chipman¹, **Tore Buchanan**², **Luis Lecuona**³ and **Charles Rupprecht**⁴

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Canine rabies has been eliminated in the US and Canada and significant strides have been made towards elimination in Mexico. Consequently in North America the spotlight has turned from managing rabies circulating in canine hosts to a focus on controlling rabies in wild carnivore and vampire bat populations to protect human and animal health and to reduce economic costs. Priority emphasis is now on arctic fox variant, raccoon variant and vampire bat variants. Oral rabies vaccination has revolutionized wildlife rabies management. Effective management targeting carnivores is now possible at the landscape scale as illustrated by the elimination of rabies in foxes in several countries in Europe and near elimination in southern Ontario. Current vampire bat control strategies to protect livestock involve vaccination and host population reduction. Research is warranted to explore more effective control strategies including oral vaccination. Key components of a wildlife rabies program include coordination, enhanced surveillance, oral vaccination, monitoring, contingency actions, applied research and adaptive management. A coordinated One Health approach balancing the art and science of wildlife management, melding traditional and non-traditional approaches and integrating aspirational with practical strategies based on sound science remains essential.

Rabies control in the 21st century necessitates clear goals developed through partnerships among bureaucrats, project managers, industry stakeholders and researchers working together to address challenges through informed risk taking and political and technical creativity. Successful management projects are characterized by sustained funding, science-based decision making, communication and a commitment to applied research as outlined in the North American Rabies Management Plan. Decision makers must have an appetite for long-term planning horizons and patience for continued support despite occasional setbacks including reemergence of previously eliminated rabies virus variants. Future success demands a better understanding of the economics of rabies control, development of next generation oral vaccines and vaccine delivery systems and a willingness to develop and adopt new technology to efficiently and effectively deliver vaccine-bait to target species populations.

ORAL VACCINATION AGAINST RABIES; POSSIBILITIES AND HURDLES BEYOND THE OBVIOUS TERRESTRIAL RESERVOIR SPECIES

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Oral rabies vaccination (ORV) has been developed into the preferred method to control and eliminate certain types of wildlife-mediated rabies, like red fox, raccoon dog, golden jackal, gray fox, coyote and raccoon. Also ORV of dogs as a supplementary tool to parenteral vaccination is suggested to increase the vaccination coverage of especially the free-roaming dog population. The utilization of ORV does not necessarily have to be restricted to these species but can be expanded to other less well known reservoir species like striped skunk, small Indian mongoose and crab-eating fox. It can also be used for conservation purposes by protecting highly endangered species from spill-over infections, like the African Wild Dog or Ethiopian Wolf. Additionally, It can be applied to protect animals that have an important economic value but cannot easily be vaccinated by the parenteral route (kudu antelope in Namibia, Africa). Furthermore, specific species that are not a reservoir species but are of concern do to their frequent human contacts could be targeted, e.g. marmosets in Brazil or temple monkeys in Thailand. Finally, ORV of vampire bats has been suggested and already investigated under experimental conditions. Besides the obvious technical hurdles like a safe and efficacious vaccine for these species, the identification of a suitable delivery vehicle (bait) and distribution system, the application of ORV for these Minor Use - Minor Species (MUMS) purposes may encounter additional obstacles. A major stumbling block is regulatory affairs; it will not always be possible to conduct the standardized safety and efficacy studies as required by the regulatory authorities (endangered and/or protected species). Hence, a more flexible stand from the authorities is required when it concerns these potential novel limited applications of ORV, like off-label use.

ROUND TABLE 6

DOG AND CAT VACCINATION STRATEGIES

Chaired by Vet. Eugenio Mirkin. Coordination of Zoonoses. National Direction of Epidemiology and Analysis of the Health Situation. Ministry of Health and Social Development, Argentina.
Co-chaired by Marco Antonio Natal Vigilato, DVM, PHS, MSc. PANAFTOSA-OPS/OMS.

Introduction: Canine rabies in the region continues to be a public health problem, due to the new cases of human rabies in recent years. During this round table, panelists share their experiences in mass vaccination of dogs for rabies. The mass vaccination of dogs continues as a valid tool in Latin America for the prevention of human cases, insofar as they take into account some important aspects: validation of the number of dogs (sampling / census) by localities, percentage of vaccine coverage reached in accordance with reality, coordination of teams in the field, improve campaign management, promote health promotion and education, increase community participation in campaigns, commitment of local and national governments, availability of resources in surveillance and laboratories, training and updating of human resources. Many improvements are needed to achieve the objectives for 2030.

Agenda and Speakers:

- 1. Challenges in canine rabies control in Guatemala, the dog population barrier.** MV David Moran. Center for Health Studies, University of the Valley of Guatemala.
- 2. Urbanization and Temporal Patterns of Dog Vaccination.** Dr. Ricardo Castillo-Neyra. University of Pennsylvania.
- 3. Improving animal-rabies surveillance activities and mass-dog vaccination campaign strategies towards canine-rabies elimination - Haiti, 2012-2017.** Melissa Etheart MD, MPH. US Centers for Disease Control and Prevention, Haiti.
- 4. The use of mobile technology in rabies mass vaccination campaigns.** Frédéric Lohr, Vet. Mission Rabies, UK.
- 5. "All models are wrong, but some are useful." Are we putting too much faith in models to plan and evaluate canine rabies interventions?** Ryan M. Wallace DVM, MPH. US Centers For Disease Control and Prevention.

CHALLENGES IN CANINE RABIES CONTROL IN GUATEMALA, THE DOG POPULATION BARRIER

David Moran¹, Danilo Alvarez¹, Loren Cadena², Julie Cleaton³, Stephanie J. Salyer², Emily Pieracci³, Ryan M. Wallace³.

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Canine rabies is endemic in Guatemala despite yearly implementation of mass vaccination campaigns targeting owned dogs in the country. Systematic evaluations of these campaigns have not been conducted by the health authorities to determine the real coverage of these campaigns. These campaigns currently report coverages of $\geq 70\%$ in most of the departments of the country based on the canine population estimation of one dog per five human inhabitants. This ratio may be under-estimating the dog population and therefore over-estimating the actual vaccination coverage, leaving the population at risk.

During October 2017 – February 2018 we conducted a sight re-sight survey on free-roaming dog populations in 19 urban, semi-urban and rural communities to estimate dog populations and the human:dog ratios in order to evaluate the accuracy of previous population estimates. Additionally, we interviewed dog owners using door to door surveys regarding ownership practices and vaccination barriers in the same communities.

The human:dog ratio varied among communities ranging from 2.7 – 14.9 (average 5.6), with the lowest ratios seen in rural communities. In the owner survey one of the most relevant findings was that 45% (419/930) of the respondents reported not knowing about the last vaccination campaign conducted in their community.

Our results show that the 75% of the owners allow their dogs roam freely, and the 5:1 ratio is not constant or in the country; therefore, the official canine population was underestimated. Consequently, the vaccination coverage was overestimated (70% vs 35%). This incorrect dog population estimation could explain persistence of rabies in areas that report coverages that are known to stop the circulation of the disease. Wide population-based studies are needed to determine accurate human:dog ratio within communities in order to plan effective rabies vaccination campaigns in Guatemala. Without accurate canine population information, eliminating canine rabies in Guatemala will be extremely challenging.

URBANIZATION AND TEMPORAL PATTERNS OF DOG VACCINATION

Ricardo Castillo-Neyra^{1,2}, Micaela De la Puente², Elvis Diaz², Katty Borrini², Amparo Toledo², Claudia Arevalo², Valerie Paz-Soldan^{2,3}, Alison Buttenheim⁴, Michael Levy^{1,2}.

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Background: Arequipa, Peru, has witnessed the first instance of reemerging canine rabies in Latin America, and the virus has been circulating in the dog population since 2014. Multiple mass dog vaccination campaigns (MDVC) per year have been conducted, but they have failed to reach the appropriate community participation and virus transmission continues.

Methods: We conducted a community-based longitudinal study for 3 years. We surveyed 4,200 houses, distributed in 5 urban localities and 6 peri-urban localities, after each annual MDVC. We conducted the surveys annually to find temporal changes in structural and behavioral barriers to dog vaccination. To follow each dog in the study area we developed a cell-phone App that retrieves dog's information and allows adding new data on follow up visits. We used a behavioral economic approach to identify behavioral bottlenecks for participating and statistical analysis to find spatial barriers and factors associated with participating in the MDVC.

Conclusions: We found suboptimal vaccination from years 1 to 3. By analyzing the temporal patterns of participation in the MDVC, we found unexpected changes at the household level. Based on their temporal patterns of participation in the MDVC, we identified four types of households: constant, intermittent, sporadic, and non-participant. This categorization will help to target communication strategies on houses and/or dog owners based on their likelihood to participate. Importantly, the distribution of the four types of household varies by level of urbanization: more constant households were found in the urban localities compared to the peri-urban localities, but houses in peri-urban localities are likely to participate intermittently in the MDVC, which offers opportunities to retain new users. We also found that participation, at the community level, might be negatively affected when the MDVC strategy is changed over time.

IMPROVING ANIMAL-RABIES SURVEILLANCE ACTIVITIES AND MASS-DOG VACCINATION CAMPAIGN STRATEGIES TOWARDS CANINE-RABIES ELIMINATION - HAITI, 2012-2017

Melissa D. Etheart, MD, MPH¹, Ludder Fleurinord², Pierre A. Dilius², Ryan M. Wallace DVM, MPH³

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Rabies is a fatal disease causing nearly 60,000 deaths globally each year; and most cases are transmitted by dogs¹. Thus a neglected disease, rabies infection is preventable. In many countries, mass-dog vaccination campaigns combined with other effective control strategies have demonstrated to reduce the transmission of rabies virus and contributed to the elimination of canine-mediated human rabies². Additionally, rabies control and elimination require effective coordination between animal health and public health systems; and must be strengthened by adequate level of: leadership, governance, political will, technical assistance, and human and financial resources. Rabies elimination activities include: education and awareness, surveillance and investigation, human and animal vaccination³.

Haiti is one of the four countries in the Western Hemisphere where canine rabies is still endemic. Haiti has an estimated of 130 human deaths annually⁴. From 2009-2012, very few bite-cases are animal-rabies cases were detected by the surveillance system in Haiti. In 2011, the Haitian Ministry of Agriculture (MARNDR) and Ministry of Health (MSPP) in collaboration with the US Centers for Disease Control and Prevention and other partners have developed the Haiti Animal Rabies Surveillance Program (HARSP) which includes: development of laboratory capacity and training of animal surveillance officers.

Since the implementation of this integrated bite-case management program more investigation of animal-bites were conducted in some regions of the country, increasing the number of reported bite-cases and rabies laboratory diagnostic. Other canine-rabies elimination efforts focused on mass-dog vaccination campaign in high-risk areas; and the implementation of alternative vaccination strategies to improve canine-rabies vaccination coverage in Haiti. Additionally, a real-time investigation tool was developed for surveillance officers.

This presentation will describe the impact of HARSP program on bite-case investigations and animal-rabies surveillance and some limitations of post-exposure prophylaxis. Additionally, we will discuss the challenges of mass-dog vaccination campaign, in Haiti.

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“ALL MODELS ARE WRONG, BUT SOME ARE USEFUL.” ARE WE PUTTING TOO MUCH FAITH IN MODELS TO PLAN AND EVALUATE CANINE RABIES INTERVENTIONS?

RYAN M. WALLACE DVM, MPH and JULIE CLEATON, MPH
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In 1996, ecologist Christopher Dye published a compartmental model, which estimated that sustained herd immunity of 39% - 57% in susceptible dog populations could eliminate rabies. When considering the confidence around these estimates, he concluded that 70% coverage would suffice in most situations. Empirical evidence over the past century has largely supported this model's findings. Other models and methodologies have since been developed, including models for dog enumeration, transmission density dynamics, impacts of population management, and many more.

All models are based upon a set of assumptions, and the reliability of the model may be highly questionable when applied to field settings where these assumptions are not satisfied. Furthermore, model output must be interpreted with attention to the “confidence” of the resulting estimates. In this presentation, we will draw upon published findings as well as field experiences in Latin America, Asia, and Africa to explore the benefits and challenges of relying upon models to develop and evaluate canine rabies elimination programs. We will discuss Dye’s definition of a “susceptible” dog, and the impact this has on planning vaccination methods. We will explore the differences in dog ownership and confinement definitions, and the resulting impact on reporting dog vaccination coverage. We will investigate assumptions associated with commonly used dog enumeration techniques, and the (often large) degree of error produced by these methods.

Lastly, we will review real-world examples where inattention to model assumptions and over-interpretation of results has impacted rabies control strategies. The presentation will conclude that models are beneficial to developing effective interventions, but only field evaluation and robust surveillance data should be used to validate the impact of a vaccination program.

SIDE MEETING

“VETERINARY INDUSTRY AND REGULATOR INTERACTIONS”

Chaired by Augusto Pich Otero, PhD - MBA. Pharmaceutical Development Manager at Pablo Cassara Laboratory SRL., Professor National at the University of La Plata, Argentina.

Co-chaired by Dr. Verónica Gutiérrez Cedillo. Ministry of Health, México.

Introduction: In this round table we'll attend different topics related to the Interaction between the Veterinary (or Animal Health) Laboratories and the Regulatory Agencies. This interaction, from the technical point of view, is critical to guaranty the Quality, Efficacy and Safety for Medicinal Products in general and, for Vaccines in particular. Also, is a key interaction for innovation activities such as new product development or new quality control methods.

Agenda and Speakers:

- 1. Analyzes of the official control of veterinary anti-rabies vaccines marketed in Brazil.** Vivian Regina Silveira Ms. Med. Vet. Laboratory of Control of Biological Products-CPB/Lanagro-SP/MAPA, Brazil.
- 2. Quality control of rabies vaccines in Argentina.** Lic. Susana Russo. National Reference Laboratory. National Service for Agri-Food Health and Quality (SENASA), Rabies Department.
- 3. Regional implementation of a potency alternative method for rabies vaccines.** Eduardo Reolón, Licentiate degree, Microbiology. Research & Development Manager, Biogénesis Bagó S.A. Argentina.
- 4. Immunity duration in primed animals. Is a single dose enough? Can it confer one-year immunity?** Diego La Torre, President of Tecnovax S.A., Argentina.

ANALYZES OF THE OFFICIAL CONTROL OF VETERINARY ANTI-RABIES VACCINES MARKETED IN BRAZIL

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The National Agricultural and Livestock Laboratories (Lanagros) are the official laboratories of the Brazilian Ministry of Agriculture. Lanagro São Paulo is responsible for the analyzes of the official control of veterinary anti-rabies vaccines marketed in the country. These official analyzes are carried out in the Laboratory of Control of Biological Products (CPB) of this Lanagro, according to the legal requirements contained in Ministerial Order no. 228 of 1988 that defines that these immunobiological must be submitted the following tests: Sterility verified in aerobic, anaerobic and fungal media; Residual Virus tested in 16 suckling mice and 20 mice (11-14 g); Safety tested in 10 mice of 14 to 16g and 05 guinea pigs of 300 to 400g; Efficiency currently proven by the NIH method, should be greater than or equal to 1 IU per dose, we used 4 groups of 20 mices (11-14g) for each vaccine, animals are vaccinated at intervals of one week, 7 days after the second vaccination are challenged; pH should be between 6.8 and 8.5. As the main goals, we have the refinement of NIH and the implementation of an alternative method, serological potency assay, to be evaluated for its performance against the different rabies vaccines we routinely test.

QUALITY CONTROL OF RABIES VACCINES IN ARGENTINA

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The Rabies Department of SENASA, the National Veterinary Service of Argentina:

- Informs the dossiers of Veterinary Rabies Vaccines
- Controls 100% of the commercial national and imported lots
- Standardizes the National Reference Vaccine (actually named DILAB PV-BHK-4) with the WHO International Standard: Sixth International Standard for Rabies vaccine
- Elaborate the rules to evaluate the Rabies Veterinary Vaccines.

The following test are performed to evaluate the biological products prior to licensing:

- Safety Control
- Sterility Control
- N.I.H. Potency Test
- Biologic Assay
- General Security
- Stability: Acelerated Thermal Degradation

Veterinary Antirrabid Vaccines controled during 2016, 2017 y 2018 (included until august) with the National Reference Vaccine DILAB PV-BHK-4.

Results

All the vaccines are inactivated and produced in cells cultures.

Safety: Satisfactory

Sterility: Satisfactory

Monodose vaccines released to the market was 99% of the dosis presented and 97.5% of the lots presented in this period and the multi-dose vaccines were 95.65% and 87.9%, respectively.

XXIX INTERNATIONAL CONFERENCE ON RABIES IN THE AMERICAS

Buenos Aires, Argentina

October 28th - November 1st 2018



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