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XXVIII

INTERNATIONAL CONFERENCE
ON RABIES IN THE AMERICAS



CANADA

INTERNATIONAL CONFERENCE ON RABIES IN THE AMERICAS

CONFÉRENCE INTERNATIONALE DE LA RAGE DANS LES AMÉRIQUES
CONFERENCIA INTERNACIONAL DE LA RABIA EN LAS AMÉRICAS
CONFERÊNCIA INTERNACIONAL DE RAIVA NAS AMÉRICAS

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XXVIII RABIES IN THE AMERICAS INTERNATIONAL
CONFERENCE

XXVIII CONFÉRENCE INTERNATIONALE DE LA RAGE
DANS LES AMÉRIQUES

XXVIII CONFERENCIA INTERNACIONAL DE LA RABIA
EN LAS AMÉRICAS

XXVIII CONFERÊNCIA INTERNACIONAL DE RAIVA
NAS AMÉRICAS

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WELCOME!

On behalf of the local planning committee, I welcome you all to the 28th International Conference on Rabies in the Americas (RITA) being held for the first time in western Canada in beautiful Calgary, Alberta, hosted by the University of Calgary Faculty of Veterinary Medicine (UCVM). Canada's newest veterinary school, UCVM has been instrumental in bringing this year's RITA conference to fruition.

The RITA meetings are essential to provide an opportunity for researchers, health professionals, international, national, provincial and local managers of rabies programs, wildlife biologists, and laboratory personnel to advance and discuss all aspects of rabies surveillance, prevention and control. We hope that this year's RITA meeting affords everyone the opportunity to create new partnerships and to renew and strengthen previous relationships between all groups working with various aspects of rabies management.

The organizers would sincerely like to thank our invited speakers, and oral and poster presenters and we appreciate the time you are taking to provide the latest information on your ongoing important rabies research. We also would like to thank all of our generous sponsors that make this event possible; the support from many different organizations has been essential to facilitating a successful, informative and enjoyable conference.

With warmest regards,

Dr Darcia Kostiuk, B.Sc.Ag., D.V.M.
Chair, RITA2017 Local Organizing Committee
Public Health Veterinarian
Government of Alberta

On behalf of the International Steering Committee, welcome to the 28th Annual International Conference on Rabies in the Americas (RITA). RITA is a major international event that provides an annual gathering of world renowned experts who will meet and share advances in rabies research and management. The meeting agenda provides many formal and informal opportunities for discussion that advance the science of rabies prevention and control. This year RITA once again includes oral and poster presentations on a myriad of topics, including the surveillance, diagnostics, pathobiology, prevention and control of rabies with a focus on humans, domestic animals and wildlife. We thank the local organizers for their outstanding efforts in establishing an exciting program in a wonderful venue and look forward to renewing long-term friendships and making new acquaintances in Calgary!

Sincerely,

Dr. Ivanete Kotait, BS, MS, DVM
President, Rabies in the Americas

BIENVENUE!

Au nom du comité de planification locale, je vous souhaite la bienvenue à la 28e Conférence internationale sur la rage dans les Amériques (RITA) qui se tient pour la première fois dans l'ouest du Canada dans la belle Calgary, en Alberta, accueillie par la Faculté de médecine vétérinaire de l'Université de Calgary (UCVM). La plus récente école vétérinaire du Canada, UCVM a contribué énormément à la réalisation de la conférence RITA de cette année.

Les réunions de la RITA sont essentielles pour permettre aux chercheurs, aux professionnels de la santé, aux gestionnaires internationaux, nationaux, provinciaux et locaux des programmes de lutte contre la rage, des biologistes de la faune et du personnel de laboratoire d'avancer et de discuter de tous les aspects de la surveillance, de la prévention et du contrôle de la rage. Nous espérons que la réunion RITA de cette année offre à tous la possibilité de créer de nouveaux partenariats et de renouveler et de renforcer les relations antérieures entre tous les groupes travaillant avec divers aspects de la gestion de la rage.

Les organisateurs aimeraient sincèrement nos conférenciers invités et les présentateurs oraux et d'affiches et nous apprécions le temps que vous prenez pour fournir les dernières informations sur votre recherche de rage. Nous tenons également à remercier tous nos généreux commanditaires qui rendent cet événement possible; le soutien de nombreuses organisations différentes a été essentiel pour faciliter une conférence réussie, instructive et agréable.

Avec mes salutations distinguées,

Dre Darcia Kostiuk, B.Sc.Ag., D.V.M.
Présidente, Comité d'organisation local de RITA2017
Vétérinaire de santé publique
Gouvernement d'Alberta

Au nom du Comité directeur international, soyez le bienvenu à la 28ème Conférence internationale annuelle sur la rage dans les Amériques (RITA). Le RITA est un événement international majeur qui offre un rassemblement annuel d'experts de renommée mondiale qui rencontreront et partageront les progrès dans la recherche et la gestion de la rage. L'ordre du jour de la réunion offre de nombreuses occasions formelles et informelles de discussion qui font progresser la science de la prévention et du contrôle de la rage. Cette année encore, RITA comprend des présentations orales et d'affiches sur une multitude de sujets, y compris la surveillance, le diagnostic, la pathologie, la prévention et le contrôle de la rage, en mettant l'accent sur les humains, les animaux domestiques et la faune sauvage. Nous remercions les organisateurs locaux pour leurs efforts remarquables dans la mise en place d'un programme passionnant dans un lieu merveilleux et nous espérons renouveler des amitiés à long terme et faire de nouvelles connaissances à Calgary!

Cordialement,

Dre Ivanete Kotait, BS, MS, DVM
Présidente, La Rage dans les Amériques

¡BIENVENIDOS!

A nombre del comité local de planificación, les doy la bienvenida a todos a la 28ª Conferencia Internacional de Rabia en las Américas (RITA) que se celebra por primera vez en el oeste de Canadá en la hermosa ciudad de Calgary, Alberta, organizada por la Facultad de Veterinaria de la Universidad de Calgary (UCVM). La más reciente escuela de veterinaria de Canadá, UCVM ha sido fundamental para llevar a buen puerto la conferencia RITA de este año.

Las reuniones de RITA son esenciales para brindar a investigadores, profesionales de la salud, gerentes internacionales, nacionales, provinciales y locales de programas de rabia, biólogos de vida silvestre y personal de laboratorio para avanzar y discutir todos los aspectos de la vigilancia, prevención y control de la rabia. Esperamos que la reunión RITA de este año proporcione a todos la oportunidad de crear nuevas alianzas y de renovar y fortalecer relaciones anteriores entre todos los grupos que trabajan con diversos aspectos de la gestión de la rabia.

Los organizadores quisieran agradecer sinceramente a nuestros oradores invitados ya los presentadores orales y de carteles y apreciamos el tiempo que están tomando para proporcionar la información más reciente sobre su importante investigación sobre la rabia. También queremos agradecer a todos nuestros generosos patrocinadores que hacen posible este evento; el apoyo un gran número de diversas organizaciones ha sido esencial para facilitar una conferencia exitosa, informativa y agradable.

Con mis mejores deseos,

Dra. Darcia Kostiuk, B.Sc.Ag., D.V.M.
Presidente, RITA2017 Comité Organizador Local
Veterinario de Salud Pública
Gobierno de Alberta

A nombre del Comité Directivo Internacional, sean ustedes bienvenidos a la XXVIII Conferencia Internacional Anual sobre la Rabia en las Américas (RITA). La RITA es un importante evento internacional que proporciona una reunión anual de expertos de renombre mundial que se reunirá para compartir los avances en la investigación y la gestión de la rabia. La agenda de la reunión ofrece muchas oportunidades formales e informales de discusión que promueven la ciencia de la prevención y el control de la rabia. Este año RITA nuevamente incluye presentaciones orales y de carteles sobre una miríada de temas, incluyendo la vigilancia, diagnóstico, pato-biología, prevención y control de la rabia con un enfoque en los seres humanos, los animales domésticos y la vida silvestre. Damos las gracias a los organizadores locales por sus sobresalientes esfuerzos en la integración de un programa emocionante en un lugar maravilloso y esperamos renovar amistades a largo plazo y hacer nuevos conocidos en Calgary!

Sinceramente,

Dra. Ivanete Kotait, BS, MS, DVM
Presidente, Rabia en las Américas

BENVINDOS!

Em nome do comitê de planejamento local, damos as boas vindas a todos à 28ª Conferência Internacional sobre Raiva nas Américas (RITA) realizada pela primeira vez no oeste do Canadá, na bela Calgary, Alberta, organizada pela Faculdade de Medicina Veterinária da Universidade de Calgary (UCVM). A mais nova escola de veterinária do Canadá, a UCVM foi fundamental para a realização da conferência RITA deste ano.

As reuniões da RITA são essenciais para permitir aos pesquisadores, profissionais de saúde, responsáveis internacionais, nacionais, regionais e locais de programas contra a raiva, biólogos da vida selvagem e pessoal de laboratório para progredir e discutir todos os aspectos da vigilância, prevenção e controle da raiva. Esperamos que a reunião RITA deste ano ofereça a todos a oportunidade de criar novas parcerias e renovar e fortalecer as relações anteriores entre todos os grupos que trabalham com os vários aspectos da gestão da raiva.

Os organizadores agradecem sinceramente aos nossos palestrantes convidados e apresentadores orais e de posters e agradecemos o tempo que você está disponibilizando para fornecer as informações mais recentes sobre sua pesquisa de raiva. Também gostaríamos de agradecer a todos os nossos generosos patrocinadores que tornam este evento possível ; O apoio de muitas organizações diferentes foi essencial para facilitar uma conferência bem sucedida, informativa e agradável.

Com os melhores cumprimentos,

Dra. Darcia Kostiuk, B.Sc.Ag., D.V.M.
Presidente do Comitê Organizador Local RITA2017
Veterinário em Saúde Pública
Governo de Alberta

Em nome do Comitê Diretor Internacional, sejam bem-vindos à 28ª Conferência Internacional Anual sobre Raiva nas Américas (RITA). A RITA é um grande evento internacional que possibilita o encontro anual de especialistas de renome mundial que se reunirão e compartilharão avanços na pesquisa e gestão da raiva. A agenda da reunião oferece muitas oportunidades formais e informais de discussão que promovem a ciência da prevenção e controle da raiva. Este ano, a RITA mais uma vez inclui apresentações orais e de pôsteres em uma miríade de tópicos, incluindo vigilância, diagnóstico, patobiologia, prevenção e controle da raiva com foco em humanos, animais domésticos e fauna selvagem. Agradecemos aos organizadores locais por seus esforços notáveis em estabelecer um programa emocionante em um local maravilhoso e esperamos renovar amizades de longo prazo e fazer novos conhecimentos em Calgary!

Atenciosamente,

Dra. Ivanete Kotait, BS, MS, DVM
Presidente, Raiva nas Américas

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GEORGE BAER LATIN AMERICAN INVESTIGATOR AWARD

PRIX GEORGE BAER LATIN AMERICAN INVESTIGATOR

PREMIO GEORGE BAER DEL JOVEN INVESTIGADOR

PRÊMIO GEORGE BAER DE JOVEM PESQUISADOR

EPIDEMIOLOGY AND MOLECULAR CHARACTERIZATION OF RABIES IN CUBA FROM 2003 TO 2016

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Introduction: Urban rabies in Cuba remains a significant problem with high levels of prevalence. Therefore, knowledge of its epidemiology is of great importance for the control of the disease.

Objective: This study was conducted in order to determine the epidemiological and molecular characteristics of rabies virus in the country.

Methods: Epidemiological study of 3347 samples from animal's brain tissue around the country during 2003 - 2016 was carried out. Samples were tested by IFD and RT-PCR assays. Sequencing and phylogenetic analysis of N gene was performed in selected 30 positive samples and the analysis of L-G region was conducted for the first time in Cuban strains.

Results: More than 15 species were under surveillance. The positivity of virus circulation throughout the country ranged from 92.31% to 19.51%. Central and western regions were the most affected with 41.9% and 53.94% respectively. Mongoose remained as the main reservoir of the virus, showing the highest rates of positivity (64.23%). It was evident a high relationship between a history of aggressive behaviour and positive results to infection (53.4%) principally in cats (64.6%). All bats that tested positive were from the *Artibeus jamaicensis* species. Phylogenetic analysis showed the presence of genotype I. All variants derived from a common ancestral. The sequence of the N region grouped the strains geographically into regional level, suggesting the existence of probable chains transmission. Terrestrial strains belonged to two different clusters. In cluster A were grouped the species from the West and East region, while in cluster B those from the East and Center region in the country. Phylogenetic analysis of the L-G gene showed a tendency to group by animal species (mongooses, dogs and cats).

Conclusion: This study showed a high prevalence of rabies in Cuba, others researches to improve the surveillance are necessary.



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**PATHOGENESIS & OTHER/PATHOGÉNÈSE et
AUTRE/PATOGENESIA y OTRO/PATOGÊNESE e OUTROS**

**ORAL PRESENTATIONS/PRÉSENTATIONS ORALES/PRESENTACIONES
ORALES/APRESENTAÇÕES ORAIS**

MODERATORS/MODÉRATEURS/MODERADORES:

Dr. Zhen Fu

Dr. Alan Jackson

RABIES VIRUS DOWN-REGULATES EXPRESSION OF EB3 AND P140CAP IN NEURONS

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Presenter: Waqas Ahmad

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Rabies virus (RABV) manifests strong affinity for the nervous system where it causes neuronal dysfunction, and alters the structural morphology of dendritic spines by inducing changes in cytoskeleton which comprises of actin, microtubule and intermediate filaments. For microtubule, EB3 is a microtubule plus-end binding protein that stabilizes microfilaments and belongs to the third family member of RP/EB group. The p140cap is an interacting partner of EB3 that together with EB3 plays vital role in postsynaptic density, dendritic spine functions and morphology. The gene expression and respective protein contents of EB3 and p140cap were compared in neuronal cells under the effect of fixed and street strain of RABV. Furthermore, gene expression levels of important actin binding, microtubule and synapse related proteins were also studied.

In this study, immunofluorescence, western blot and Real time PCR were carried out to compare the gene expression and corresponding protein contents of different cytoskeleton related proteins. Both strains of RABV significantly reduced the gene expression and protein contents of EB3 and p140cap. However, the street strain considerably inhibited the transcription level of p140cap, but had no significant effect on its protein level. The fixed strain produced fractured microtubules in fixed neurons, and down-regulated different microtubule and actin associated proteins, while up-regulated the level of Tesk2. In neuronal cells, the fluorescence localization of EB3 protein was random and varied at 48 hour and 98 hours of post-infection.

Like other neurodegenerative diseases, rabies may possibly alter the neuronal structures by changing the gene expression levels of integral protein binding partners of microtubules and actin. Most importantly, EB3 and p140cap are vital in maintaining the morphology of dendritic spine. The results could be utilized to envisage other neurological diseases by explaining the intricate relationship between EB3 and p140cap in dendritic spines.

RABIES DERIVED PEPTIDES INTERACT WITH NEURONAL NICOTINIC RECEPTORS AND ALTER ANIMAL BEHAVIOR

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Many infectious disease agents, including rabies virus, alter host behavior in order to aid their transmission and maintenance. Rabies virus glycoprotein contains a neurotoxin-like 29 amino acid motif that interacts with nicotinic acetylcholine receptors (nAChRs) at the neuromuscular junction. These neurotransmitter receptors are also prevalent within the central nervous system. In this project, we test if the rabies virus glycoprotein interacts with neuronal neurotransmitter receptors in the CNS and if this interaction plays a role in modulating host behavior. We show that both the rabies-derived peptide and the full-length ectodomain bind to and inhibit the function of CNS alpha4 beta2 nAChRs. The neurotoxin-like peptide inhibits pharyngeal pumping in *C. elegans*, a behavior initiated by nAChRs. Peptide injected into the cerebrospinal fluid of mice produce hyperactivity. Results suggest that this domain of the rabies virus glycoprotein can interact with an important group of neurotransmitter receptors in the central nervous system, and that this interaction contributes to the drastic behavioral changes seen in rabies-infected animals.

ACTIVATION OF DENDRITIC CELLS IS ASSOCIATED WITH THE GLYCOSYLATION PATTERN OF RABIES VIRUS GLYCOPROTEIN

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Dendritic cells (DCs) are antigen-presenting cells of the mammalian immune system and act as messengers between the innate and the adaptive immune systems. Our previous studies have shown that laboratory-adapted rabies viruses (RABV) can activate DCs. On the other hand, wild-type RABVs cannot efficiently activate DCs. Using recombinant RABVs with exchange of the glycoprotein (G) between the laboratory and wild-type RABVs, it was found that it is the G that determines the ability of a given RABV to activate DCs. Recombinant chimeric RABV viruses were further constructed by exchanging each of the structural domains of the G, i.e. signal peptide, ectodomain, transmembrane, and cytoplasmic tail between laboratory-adapted and wild-type RABVs. Viruses containing the ectodomain of the G from laboratory-adapted, but not that from wild-type, RABV was found to activate DCs. Further mapping of ectodomain by mutational analysis indicate that the presence of additional glycosylation site on the G from the laboratory-adopted RABV is responsible for efficient DC activation. Together, our results demonstrate that G-mediated DC activation is associated with the glycosylation pattern of RABV G protein.

RABIES IS REAL - AN INNOVATIVE APPROACH TO RABIES AWARENESS

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In the mid-1980's, Ontario was known as the "Rabies Capital of North America". Due to the Ontario Ministry of Natural Resources and Forestry's successful vaccine baiting program, rabies cases declined dramatically post 1989. Further, Raccoon Rabies strain was eliminated in Ontario in 2007 after an outbreak in eastern Ontario from 1999 to 2005, plus cases from other rabies strains in terrestrial animals continued to decline across Ontario. Concurrently, the public's knowledge about rabies appeared to decline; people wanted to feed, own, or help sick or injured animals including wildlife. Concern about these behaviours rose when the Raccoon Rabies outbreak was identified in Hamilton December 2015. This marked the first case of any terrestrial rabies in Hamilton since 1994. It was anticipated the community was naïve about the rabies risk from wildlife, needed to be educated and informed about rabies and the potential fatal outcome. Hamilton Public Health wanted a rabies campaign that was innovative, unique, and would capture people's attention motivating them to learn more about rabies. A campaign was developed that embraces creativity, humour, and sparks curiosity to help educate the public about the seriousness of rabies. Whimsical, imaginary animals were used and the main theme is Rabies is Real. The campaign was focus-tested and to date is well received by the public, stakeholders, and partners and was adopted by several Ontario health units. Various campaign products were produced including videos, posters, social media ads, and factsheets. Since the

outbreak and campaign, Hamilton Public Health's main rabies webpage saw hits rise from 500 to nearly 7000 visits annually. The evaluation of the campaign and response to the outbreak will conclude in 2017. Work continues for the next three to five years with partners and stakeholders to control and eliminate the outbreak, while these products continue communicating Rabies is Real.



HUMAN RABIES AND PROPHYLAXIS/LA RAGE HUMAINE ET LA PROPHYLAXIC/RABIA HUMANA Y PROFILAXIS/RAIVA HUMANA E PROFILAXIA

ORAL PRESENTATIONS/PRÉSENTATIONS ORALES/PRESENTACIONES
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MODERATOR/MODÉRATEUR/MODERADOR:
Dr. Alan Jackson

PROTECTIVE EFFICACY OF MONOCLONAL ANTIBODY R172 AGAINST NORTH AMERICAN RABIES VIRUS VARIANTS IN THE HAMSTER LETHAL CHALLENGE MODEL OF POST-EXPOSURE PROPHYLAXIS (PEP)

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The diversity of North American rabies variants requires that rabies biologics demonstrate broad neutralizing activity. RAB1 and RAB2 are human monoclonal antibodies recognizing distinct rabies glycoprotein epitopes. R172 (RAB1 and RAB2 combined) has shown in vitro neutralization of a broad panel of North American isolates with six variants identified with alterations in residues critical for RAB1 or RAB2 binding. In a PEP model, R172 neutralization of three variants was evaluated: a Texas coyote isolate (cosmopolitan dog variant), an Arizona *Parastrellus hesperus* bat isolate (Ph bat variant) and an Arizona grey fox isolate from a host-shift event from *Eptesicus fuscus* bats (Ef-w1 variant). For each PEP experiment, groups of 12 or 21 Syrian hamsters were inoculated intramuscularly with rabies virus. Passive antibody was administered at the inoculation site 24 hours post-exposure with rabies vaccine administered in the contralateral limb. Vaccination was repeated on days 3, 7, 14, and 28; animals were observed for 45 days. Controls received no treatment or vaccine only. Three doses of R172 (0.01 mg/kg, 0.1 mg/kg, 1.1 mg/kg) were compared to a 20 IU/kg HRIG PEP regimen. All controls died in the cosmopolitan dog and Ph bat variant experiments, whereas 5% of untreated and 10% of vaccine only controls survived in the Ef-w1 study. Survival in HRIG PEP cohorts ranged from 61% - 75%. No significant survival differences were observed between the R172 and HRIG cohorts challenged with cosmopolitan dog variant. Significantly higher survival was observed in the mid (100%) and high dose (100%) R172 groups than the HRIG cohort (62%) challenged with Ph bat variant ($p < 0.01$) and in the Ef-w1 study, 95% survival occurred for mid and high dose cohorts compared to 61% for the HRIG cohort ($p = 0.02$). These data demonstrate that R172-containing PEP provides in vivo protection that equals or exceeds protection elicited by HRIG-containing PEP for North American dog and bat rabies virus variants.

DEVELOPMENT OF ANTI-RABIES MABS FOR POST-EXPOSURE PROPHYLAXIS

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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. Results from these clinical trials will be presented.

CLINICAL STUDY OF THE SAFETY AND EFFECTIVENESS OF SIMULATED POST-EXPOSURE PROPHYLAXIS WITH HUMAN IMMUNE GLOBULIN (KEDRAB) WITH CO-ADMINISTRATION OF ACTIVE VACCINE IN HEALTHY SUBJECTS

Mark Matson, Eran Schenker, Michal Stein, Vladislava Zamfirova, Garrett E. Bergman

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Kamada Ltd. and Kedrion conducted a prospective, randomized, double-blind, single-period, non-inferiority and safety study comparing investigational rabies immune globulin (Kamada-HRIG; KedRAB®) with a marketed rabies immune globulin (HRIG Comparator, HyperRAB®). A total of 118 randomized healthy, previously unvaccinated adult subjects received one dose (20 IU/kg) of KedRAB or HRIG Comparator on Day 0. All subjects were to receive rabies vaccine (RabAvert®; 1 mL of ≥ 2.5 IU/mL) on Days 0, 3, 7, 14, and 28. Follow-up visits occurred on Days 49 and 185. Efficacy was defined as the proportion of subjects receiving KedRAB and achieving Rabies Virus Neutralizing Antibodies (RVNA) titer of ≥ 0.5 IU/mL (guidelines recommended, putatively therapeutic titer) at 14 days after IM injection. Almost all subjects in the KedRAB group (55 of 56 subjects; 98.2%) and all subjects in the HRIG Comparator group developed an RVNA titer ≥ 0.5 IU/mL on Day 14 demonstrated by rapid fluorescent focus inhibition test. The difference between the proportion of subjects with RVNA titer ≥ 0.5 IU/mL on Day 14 in the KedRAB and HRIG Comparator groups was -1.8% (90% CI: -8.2, 3.1). The lower limit of the 90% CI was greater than the pre-specified non-inferiority margin of -10%, thus demonstrating that KedRAB was non-inferior to HRIG Comparator for the primary endpoint. KedRAB was well tolerated, demonstrating a safety profile comparable to the HRIG Comparator, with the exception of injection site pain, which was reported more frequently. There were no meaningful differences between the groups regarding treatment emergent adverse events, laboratory values, vital signs, or ECGs. This result indicated that KedRAB provides a protective level of neutralizing anti-rabies antibodies comparable with that of the HRIG Comparator, when either is given in conjunction with rabies vaccine. Further study of safety and efficacy in pediatric patients is underway.

VACCINE HESITANCY AND RABIES DEATHS AMONG PREGNANT AND BREASTFEEDING WOMEN IN VIETNAM, 2015– 2016

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Introduction: Human rabies deaths are preventable through prompt administration of post-exposure prophylaxis (PEP) after exposure to rabid animals. PEP (immunoglobulin and vaccine) is safe for all segments of the population. Vietnam has made monumental progress towards reducing human rabies deaths. Within a 22 year time frame, cases have dropped 82%, from 505 cases in 1994 to 91 cases in 2016, largely due to the expansion of PEP facilities within the past decade. Despite widespread availability of PEP in Vietnam, the Ministry of Health (MoH) received reports of pregnant and breastfeeding women clinically diagnosed with rabies in 2015.

Methods: The MoH reviewed the case investigation forms of these probable cases (clinical definition and documented animal exposure) to identify factors associated to their deaths.

Results: During 2015–2016, six probable rabies cases occurred among pregnant or breastfeeding women. The median age was 27 years (range: 19–33), and all case-patients attended a minimum of junior high school. Two (33.3%) of the case-patients sought care from a medical provider or traditional healer. None of the case-patients sought PEP after being bitten by unvaccinated dogs. All families reported the case-patients' fear of adverse events from PEP to their fetus or child as the primary barrier to them receiving PEP. Four (67%) case-patients were pregnant during their rabies exposure. Three (75%) were 32–37 weeks pregnant during symptom onset, prompting emergency cesarean section. One of the three neonates died from complications unrelated to rabies.

Conclusion: This investigation uncovered a series of six rabies deaths among breastfeeding or pregnant women that appear to be associated with vaccine hesitancy due to limited knowledge about rabies risk and treatment options. These findings highlight the need for targeted messaging about the safety and effectiveness of PEP for vulnerable populations to medical providers, traditional healers, and pregnant and breastfeeding women.

EVALUATION OF THE EXPECTED COSTS AND BENEFITS OF INCREASING ACCESS TO HUMAN RABIES BIOLOGICS

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Presenter: Ryan Wallace

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Rabies deaths are preventable with timely application of rabies post-exposure prophylaxis (PEP). Despite tens of millions of PEP doses used annually, still an estimated 59,000 deaths occur. The majority of rabies deaths are attributed to lack of healthcare seeking behavior by the bite victim, which is often attributed to lack of money, lack of knowledge, and lack access to PEP centers. Clearly, improving PEP access through reduced costs and increased access would result in reduction in global rabies deaths. However, recent studies have shown that dog bite events are common in most communities, and the rate of rabies in biting dogs is relatively low. Therefore, a majority of rabies PEP is likely provided unnecessarily. At an estimated global average of \$100 per PEP regimen, unnecessary PEP provision constitutes a substantial cost burden, which would be increased through indiscriminate improvements in access. CDC developed an economic model that uses probabilities of exposure and healthcare seeking behaviors to estimate the cost and human deaths averted through increased PEP accessibility. Using Haiti parameters, this model suggests that the indiscriminate increase in PEP for bite victims from an availability rate of 25% to 50% would lead to an increase in PEP cost of \$257,064 while potentially preventing an additional 16 human rabies cases (cost per additional death averted of \$16,067). However, when PEP is provided based upon a risk assessment, and PEP is delayed for low-risk exposures, the same increase in access would lead to an increased in cost of \$9,145 while reducing the national burden by 16 human rabies deaths (cost per additional death averted of \$571). We provide a method to quantitatively assess the cost-benefit of scaling up access to rabies PEP. Our results highlight the importance of increasing access with consideration to the exposure risk.

RETROSPECTIVE REVIEW OF HUMAN RABIES CASES IN CANADA

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No complete record of human rabies cases in Canada currently exists in the published literature. This is despite rabies being reportable under the Canadian National Disease Surveillance System (CNDSS) since 1927. A retrospective review of published literature, technical government reports, and national archives of newspaper articles was conducted to describe the number and nature of human rabies deaths in Canada. Epidemiological details including the animal species causing infection, location and type of exposure (bite vs non-bite), patient's age and sex, clinical symptoms and time from exposure to symptoms and death were also collected. We found 50 documented human rabies cases in Canada between 1814 and 2012, the last reported human rabies death in Canada. We identified 24 cases since the CNDSS began reporting in Quebec (11), Ontario (8), Alberta (2), Saskatchewan (1), British Columbia (1), and Nova Scotia (1). Two cases were acquired internationally, and the remaining 22 were acquired domestically. Seventy-five percent (18/24) of cases had details of the exposure incident. Out of these cases, 13 males and 5 females were identified with ages from 3 to 73. Bite exposures occurred in 15 cases primarily to the face, arms, and hands (11/24). Dogs (7/24) and bats (6/24) were the most common animal species causing infection. Beginning in the 1960s, there is a marked shift away from canine to wildlife-associated rabies, with the last 6 domestically acquired cases due to incidents involving bats. This shift corresponds with the introduction and availability of companion animal rabies vaccines during the 1940s and 1950s. This retrospective review is the first detailed summary of human rabies cases in Canada that provides insight into how historical human rabies deaths were related to rabies outbreaks in dogs, and the impact of rabies management actions, such as human and animal vaccine developments and practices, over time.



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VACCINES AND ANTIVIRALS/VACCINS ET
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ORAL PRESENTATIONS/PRÉSENTATIONS ORALES/PRESENTACIONES
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MODERATORS/MODÉRATEURS/MODERADORES:

Dr. Christine Fehlner-Gardiner

Dr. Julio Pompei

MONITORING THE CANINE VACCINE COLD CHAIN IN THE FIELD

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The World Health Organisation stresses the importance of maintaining the cold chain for temperature sensitive vaccines. Due to the recommendation for rabies vaccines to be stored between 2° and 8° Celsius and the nature of canine mass vaccination projects, cold chain maintenance remains one of the most significant logistical hurdles project managers have to overcome. Mission Rabies field teams in Malawi evaluated the use of adhesive temperature monitors as an alternative to thermometers in both storage fridges and field cooler boxes during the duration of a 4 week mass vaccination drive in April/May 2016. Two different Timestrip® temperature monitors were used to alert the teams to the critical temperature thresholds of 0° C and 30° C. As freezing rapidly damages vaccines, each vaccine box containing 10 vials of 10ml vaccine was marked with a monitor that indicates the immediate crossing of the lower threshold through colour change. As damage from high temperatures is related to both the temperature achieved and the time of exposure, to test for the upper temperature limit each cooler box was equipped with a monitor that showed the crossing of the upper threshold over time. Of a total of 22 vaccination teams participating in the study, 7 teams reported an exposure of the vaccines to temperatures above 30° C for more than 4 hours and on 9 occasions teams reported a temperature drop to 0° or below. These findings highlight the importance of monitoring the temperature during field campaigns, both due to proximity to ice packs and exposure to high outside temperatures. The use of Timestrip temperature indicators gives early visual clues and raises awareness among the team. However practicability of the temperature monitoring must be taken into consideration and adhesive monitors need to be improved with regards to their setup to make them a valuable tool in field projects.

NOVEL 3-DOSE RABIES VACCINE BASED ON RECOMBINANT G PROTEIN NANOPARTICLES

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Rabies remains an important public health threat in most developing countries. Currently available Rabies vaccines require five injections spread over 28 days, which is very difficult to comply with, in practice. As a result, ~40% patients receive four or fewer intramuscular injections instead of five¹⁻². Data from 215 vaccine recipients revealed that compliance drops from 87.9% on Day 7 to 60% on Day 28. The Rabies Glycoprotein (G-protein) is an important surface protein central to the disease process. We first identified the genetic sequence of the G-protein, and then employed sophisticated recombinant techniques to clone the gene into the BV. Correctly folded Rabies G-proteins are then extracted from the cell surface and purified to maintain their three-dimensional structure and biological activity, ultimately serving as the immunogenic molecule in our vaccine. The vaccine has demonstrated very high potential for success in Phase II human clinical trial. Phase II clinical trial was conducted in 225 subjects. The vaccine showed better sero-protective titres starting from day 14 to day 270 with 3 doses of Rabies G protein vaccine over schedule of 0-3-7 compared to reference vaccine. The 3 dose vaccine would ensure significantly higher compliance to the complete vaccine regimen as described above.

1. Sudarshana et al. *Int J Infect Dis* 2007 11 : 29-55
2. Shankaraiah, et al.: Compliance to anti rabies vaccination. *IJPH*;59, Issue 1, January- March, 2015

RISK ASSESSMENT FOR POTENTIAL SHEDDING OF AN ORAL RABIES VIRUS VACCINE

Ad Vos, Conrad Freuling, Steffen Ortmann, Antje Kretzschmar, Jeannette Kliemt, Annette Schliephake, Volker Herwig, Thomas Müller

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As part of the safety evaluation of candidate oral rabies vaccines spread and dissemination of the vaccine strain should be investigated in detail, especially in saliva and feces. The presence of vaccine virus in these secretions could result in direct and indirect transmission to conspecifics and non-target species. Hence, the shedding (saliva, feces) of the candidate vaccine strain SPBN GASGAS has been investigated in 8 different target and non-target species; red fox, raccoon dog, small Indian mongoose, raccoon, striped skunk, domestic dog, domestic cat and domestic pig. Samples collected at different time points after oral administration of the vaccine strain were examined for the presence of viral RNA (rt-PCR) and infectious virus (RTCIT); if PCR-positive, the samples were subsequently investigated by RTCIT. No infectious virus was detected in any of the feces samples examined at any time post vaccine administration. Also, RNA-fragments were detected in a relatively low percentage of the feces samples; indicating rapid degradation of the virus in the gastro-intestinal tract. In contrast, RNA-fragments were detected in the saliva swabs for extended periods (up to 10 days) after vaccine administration, but viable virus was only present in the samples taken during the first hours post vaccine administration. The virus detected during the first couple of hours post vaccine administration is not a result of active replication of the vaccine virus in the host but is virus administered but not yet taken up or cleared. Hence, it can be concluded that there is no active shedding of the vaccine virus SPBN GASGAS after oral administration. Therefore, the potential risk of horizontal transmission of the vaccine virus under field conditions is restricted to the first couple of hours after vaccine bait uptake.

NEUTRALIZATION OF DIVERGENT LYSSAVIRUSES WITH AVAILABLE BIOLOGICS IN VITRO

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Divergent viruses of the genus *Lyssavirus* include Shimoni bat lyssavirus (SHIBV), Mokola lyssavirus (MOKV), Lagos bat lyssavirus (LBV), West Caucasian bat lyssavirus (WCBV), Ikoma lyssavirus (IKOV), and Lledia bat virus (LLEBV). These viruses can cause acute encephalitis in animals indistinguishable from rabies, and MOKV has been implicated in human deaths. Except for MOKV, the public health impact of these viruses remains unknown. Based on variation in the glycoprotein and animal studies, the conclusion has been that available rabies biologics do not offer protection against these viruses. We tested three lots of human rabies immune globulin (HRIG) and six lots of intravenous immune globulin (IVIG), available from commercial providers in North America, using a standard rapid fluorescent focus inhibition test method. All of the divergent lyssaviruses, except LLEBV of which an isolate is not available, were adapted to the test. All of the lots of HRIG tested neutralized SHIBV, MOKV, LBV, and WCBV. At least one lot of HRIG neutralized IKOV; although, neutralization was only observed at 10^{-0.3}, lower than the standard starting dilution of 10^{-0.7}. All lots of IVIG from one source neutralized LBV and WCBV at low dilutions. These results suggest that currently available rabies specific polyclonal immune globulins have some neutralizing potential against divergent lyssaviruses, and that post-exposure prophylaxis for laboratory exposure to divergent lyssaviruses may be effective. Additional testing will be done in the future to increase evidence for this observation and further investigate the activity of rabies biologics against divergent lyssaviruses in vitro as well as in vivo.

PRIMARY RABIES PRE-EXPOSURE VACCINATION AND BOOSTER RESPONSE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Objective: A systematic review and meta-analysis was conducted to synthesize the current evidence available in the literature with a focus on rabies virus neutralizing antibody response (as measured by seroconversion rates (SCR) and geometric mean titers (GMT)) generated by different vaccines, administration routes, doses, and schedules.

Design: The MEDLINE, EMBASE, Cochrane Library, and WHO Index Medicus databases were systematically searched. After a primary screening, 120 articles were identified for further review, of which 51 (42.5%) met inclusion criteria. Critical review and data collection was conducted by two reviewers and entered into a database for analysis.

Results: The intramuscular (IM) vaccine administration route showed relatively little variation in primary SCRs from different administration routes and schedules compared to the intradermal (ID) vaccine route. In general, the ID route had slightly lower estimated SCRs after primary vaccination. However, all study cohort subjects responded to a booster vaccination or simulated postexposure prophylaxis regardless of vaccine, route, schedule, length of time since primary vaccination, or titer at time of booster.

Conclusions: Primary SCRs were robust when administered by the IM route regardless of deviations from current recommendations. Multi-site ID regimens may be preferred to ensure complete SCRs and to maintain higher GMTs for a longer duration of time. Furthermore, given the robust response to booster vaccination, future evaluations should examine the objectives of routine serological monitoring for risk groups and if currently recommended frequencies and cut-offs are cost effective or necessary.

PARTIAL STABILITY AND QUALITY ATTRIBUTES ASSESSMENT OF ACTIVE PHARMACEUTICAL INGREDIENT AND LYOPHILIZED RABIES VACCINE

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The stability of vaccine preparations during their period of use is an essential aspect to confirm during the development and before commercialization, demonstrating the thermostability, immunogenicity and protectiveness of the product in potency assays. In previous reports, we have demonstrated a consistent rabies vaccine production bioprocess, complying with the European Pharmacopeia (Ph. Eur.) in terms of purity, safety and immunogenicity. The present work is aimed to show the in-course stability assessment of three different lots of the active pharmaceutical ingredient (API) at $5\pm 3^{\circ}\text{C}$, and their corresponding final lyophilized products at $5\pm 3^{\circ}\text{C}$ and 37°C . The potency was determined by the NIH mouse rabies challenge assay (NIH test), which is one of the selected stability parameter. Other relevant quality attributes were also evaluated, which also play a significant role on the stability of the vaccines, as the glycoprotein content by ELISA, residual moisture content (RMC) by gravimetric Karl Fischer method, and pH when reconstituted. Appearance and color were also visually monitored. Additionally, the assessment of the stability in different bioprocess intermediates (harvest, purified virus and final bulk). The partial results indicate that, up to 6 months, two of the three API lots showed potency values, glycoprotein content, appearance and pH, within the product specifications. The lyophilized vaccine resulted stable, yielding potency values higher than 2.5 NIH IU per dose, for both temperature conditions. The RMC in the final product was $\leq 3\%$, and the appearance was accepted as a white to off-white lyophilized powder and a clear solution when reconstituted. Finally, the intermediate analysis allowed us a better process design, determining the appropriate storage conditions at each step. The stability and quality attributes of the API and the lyophilized rabies vaccine, showed promissory results representing an essential information to obtain the final product registry.



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**DOMESTIC ANIMAL RABIES/RAGE DES ANIMAUX
DOMESTIQUES/RABIA ANIMAL DOMÉSTICA/RAIVA
ANIMAL DOMÉSTICA**

**ORAL PRESENTATIONS/PRÉSENTATIONS ORALES/PRESENTACIONES
ORALES/APRESENTAÇÕES ORAIS**

MODERATORS/MODÉRATEURS/MODERADORES:

**Dr. Catherine Filejski
Dr. Juan Montano Hirose**

MICROPLANNING OF URBAN CANINE RABIES VACCINATION CAMPAIGNS, HAITI, 2017

Benjamin Monroe, Fleurinord Ludder, Andy Gibson, Pierre Dilius, Kelly Crowdis, Franso Fracciterne, Frederic Lohr, Julie Cleaton, Jesse Blanton, Marco Vigilato, Max Millien, Ryan Wallace

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Haiti has the highest estimated human rabies burden of any country in the Western Hemisphere. While the country has recently made significant strides combating rabies through strengthening surveillance capacity and investing in nationwide canine vaccination campaigns, numerous logistical obstacles have prevented Haiti from reaching the internationally recognized goal of 80% canine vaccination coverage. These difficulties include inaccurate population estimates, a deficit of trained vaccinators, densely packed cities, populations in remote mountainous areas, and poor infrastructure for communication and transportation. In 2017, a collaboration between the Ministry of Agriculture of Haiti, the United States Centers for Disease Control and Prevention, the Pan American Health Organization, Mission Rabies, and Christian Veterinary Mission has led to a data-driven strategy for vaccination of 800,000 dogs in Haiti. The approach uses a smartphone application to direct trained vaccination teams to specific priority areas where they systematically vaccinate all available dogs and record the location information. The data is uploaded to a central repository and analyzed daily so teams can be directed to move to a new area once a threshold is reached. The newly-established system was evaluated in two urban areas of Artibonite Department in May 2017. The cities were divided into 69 zones of approximately 500 dogs with recently developed human-dog ratios, census data, and satellite imagery. The cities were split geographically into intervention and control areas. Twenty-three zones were randomly selected for evaluation with in person interviews and dog site-resite studies. Teams vaccinated 10,472 dogs over 12 vaccination days with coverage of 77% in the intervention areas compared to 40% in control areas. The lessons learned in this two-week campaign will be used to develop a national canine rabies vaccination plan.

UTILITY OF A PHOTO BASED SIGHT-RESIGHT STUDY IN A DOG POPULATION IN HAITI, 2016

Julie Cleaton, Ryan Wallace, Benjamin Monroe

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Accurate local and national dog population estimates are important for successful rabies vaccination campaign planning and analysis, but these numbers are rarely available for canine variant endemic countries. In order to assess population size and vaccination coverage, a photo-based sight-resight study was performed following a vaccination campaign in August 2016. Two field evaluators walked along tracks in designated areas on two consecutive days, photographing each dog seen and recording vaccination marks, sex, body condition score, color, wounds, distinctive marks, and sight or resight status. Two CDC evaluators compared the photos from both days and determined sights and resights, confirming with each other and the field records when quality photos were missing. Resighted dogs were then matched and compared for collar loss and reviewer concordance.

Comparing determination of sight versus resight using Cohen's kappa, field team concordance to the final evaluation was only 0.29, whereas CDC reviewer concordance between each other was 0.44 and as a team to the final evaluation was 0.73. Concordance between the CDC team and the field team was lowest at 0.04. For total population estimates, the field team underestimated the final evaluation by 5%. The average human to dog ratio across all 5 sites was 13.9 before corrections and 10.3 after accounting for confined dogs. The vaccination coverage among unique dogs was 57% before corrections and 61% after accounting for collar loss. 13.7% of dogs were recorded to have a mark or collar on day 1 and were not recorded to have one on day 2. General population estimates from the field and the photo-enhanced totals were very close, but the field team usually underestimated and did not consistently select the correct dogs as resights. The photo-based method allowed us to verify the field estimates and assess collar and wax mark loss among vaccinated dogs.

RABIES ANTIBODY LEVELS IN IN DIFFERENT GROUPS OF PETS FOLLOWING VACCINATION

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Currently, rabies serology testing utilized as a check of immunization is of interest to those traveling with their animal companions; along with those uneasy with over-vaccinating animals. The Kansas State University Veterinary Diagnostic Laboratory (KSVDL) Rabies Laboratory, an OIE (World Organisation for Animal Health) approved laboratory, has provided serological rabies testing for pet travel purposes since 2002 by two recognized testing methods: Fluorescent Antibody Virus Neutralization (FAVN) and Rapid Fluorescent Focus Inhibition Test (RFFIT). 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 may be concerned about vaccine booster effects on health status of the pet. Due to the number of samples tested for both these groups, cumulative amounts of data is now available to analysis rabies antibody levels in companion animals. Though policies vary between quarantine stations of rabies-free destinations, a majority requires a minimum rabies antibody level greater than or equal to 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 vaccinated. Analysis of the dataset identified a difference in percent of dogs and cats with results below 0.50 IU/mL for pets traveling and not traveling with 3-5% for traveling pets and 13-15% for those not traveling. Thus, indicating a positive effect of intentional timing of vaccination and blood draw portrayed in the measured antibody response to rabies.

TOWARDS CANINE RABIES ELIMINATION IN MEXICO: NEW CHALLENGE REVEALS IN 2017

Nidia Aréchiga Ceballos*, Susana Chávez López, Albert Sandoval Borja, Martín Melo Munguía, Mauricio Gómez Sierra, David Martínez Solís, Rita Terán Toledo, Israel Animas Vargas, Beatriz Escamilla Ríos, Arafat Animas Vargas, Miriam Denisse Fragoso Fernández, Mauricio Vázquez Pichardo, Belém Torres Longoria, Irma López Martínez and Alberto Díaz Quiñónez.

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In the last years in Mexico, canine rabies cases with antigenic variant 1 (AgV1) were restricted to the states of Yucatan and Chiapas. Since 2011, dog cases in Chiapas have this variant while in Yucatan an atypical antigenic reaction pattern (AAgR) was detected. Viruses with this AAgR are phylogenetically clustered in a lineage of wild-living mammals. Although the reservoir species could not be identified, skunks have an important role as transmitters to other species. In 2017, urban rabies was present in one dog and cats from Yucatan, one dog from Chiapas and one from Sinaloa. In Yucatan one puppy and a litter of kitten (n=4), none of them vaccinated, were positive to rabies. The dog had AgV3 related to hematophagous bats and the kittens had AAgR. The phylogenetic reconstruction grouped them in the lineage of viruses related to wild rabies cycle. In Chiapas one non-vaccinated puppy harbored the same AgV3. Surprisingly, in July a non-vaccinated dog from Sinaloa, where canine rabies has not been reported since many years, had AgV1. The sequence of this virus confirmed that this is not related to the typical canine virus that has been eliminated in this state by vaccination campaign but it belongs to the previously reported lineage of skunks in Chihuahua, Sinaloa, Sonora and Durango that harbored this virus that antigenically cannot be differentiated with monoclonal antibodies but genetically it belongs to a different lineage. Later it was confirmed that this dog was attacked by skunk. These analyses confirmed that classical AgV1 has been controlled by vaccination and reveals a new challenge: new born non-vaccinated dogs and cats susceptible to rabies virus in places in which urban and wild cycle rabies overlaps.



BAT RABIES/RAGE DE CHAUVÉ-SOURIS/RABIA DE MURCIÉLAGO/RAIVA DO MORCEGOS

ORAL PRESENTATIONS/PRÉSENTATIONS ORALES/PRESENTACIONES
ORALES/APRESENTAÇÕES ORAIS

MODERATORS/MODÉRATEURS/MODERADORES:
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THE CONTINUING CHALLENGE OF NOVEL LYSSAVIRUSES

Ashley C. Banyard, Denise Marston, David Selden, Guanghui Wu, Conrad Freuling, Stefan Finke, Thomas Muller, Juan Echevarria Anthony R. Fooks

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The continued discovery of lyssaviruses is of great importance to both medical and veterinary fields. Whilst rabies virus causes over 59,000 deaths every year, all lyssaviruses are capable of causing a fatal encephalitis that is clinically indistinguishable from rabies. In the last 10 years a number of novel lyssaviruses have been characterised from wildlife, predominantly bats species. These viruses, whilst not thought to directly pose a threat to human or animal health remain of high consequence because of the fatal outcome predicted following development of clinical disease. The lyssaviruses have been tentatively classified into phylogroups according to phylogenetic and antigenic features that have been used to differentiate them. Lyssaviruses have been tentatively classified into three phylogroups with phylogroups I and II being relatively well defined and a further third phylogroup, containing the most divergent lyssaviruses, requiring more investigation to understand the relationships between these viruses. Importantly, the current rabies vaccines afford protection against phylogroup I lyssaviruses although the serological titre required to enable this anti-glycoprotein based antibody neutralisation has not been defined for each lyssavirus species. Further, this rabies vaccine generated neutralising antibody response is unable to extend protection against phylogroup II and III viruses. Most recently, two novel lyssaviruses, Gannoruwa bat lyssavirus and Lleida bat lyssavirus have been discovered in bat species and here we describe the in vitro characterisation of these viruses and demonstrate peripheral pathogenicity in mice. Further we assess the ability of existing rabies vaccines to enable protection from these viruses using an in vivo vaccination challenge model and demonstrate that although vaccines are able to protect against Gannoruwa bat lyssavirus, no protection is afforded to the more divergent Lleida bat lyssavirus.

LONG-TERM POPULATION SURVEYS OF TWO SEROTINE BAT (*EPTESICUS SEROTINUS*) COLONIES EXPOSED TO EBLV-1 (EUROPEAN BAT LYSSAVIRUS TYPE 1): ASSESSMENT OF RABIES TRANSMISSION USING CAPTURE-RECAPTURE MODELS

Emmanuelle Robardet, Christophe Borel, Marie Moinet, Dorothée Jouan, Marine Wasniewski, Jacques Barrat, Franck Boué, Elodie Montchâtre-Leroy, Alexandre Servat, Olivier Gimenez, Florence Cliquet, Evelyne Picard-Meyer

Presenter: Emmanuelle Robardet

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This study describes 2 longitudinal serological surveys of serotine bat (*Eptesicus serotinus*) parturition colonies located in the North-East of France. This species is currently considered as the main EBLV-1 reservoir. Multievent capture-recapture models were used to determine the factors influencing bat rabies transmission as this method accounts for imperfect detection and uncertainty in disease states. Considering the period study, analyses revealed that survival and recapture probabilities were not affected by the serological status of individuals, confirming the exceptional capacity of bats to be exposed to rabies viruses without dying or even showing clinical signs of the disease. Bats have been observed to both seroconvert (i.e. move from a negative to a positive serological status) and serorevert (i.e. move from a positive to a negative serological status). Indeed, the seroreversion frequencies of adults were higher than other transition states in juveniles. This might suggest that the study was carried out at the end of the epidemic. On one of the 2 sites, temporal dynamics of the infection show a constant decrease in seroprevalence throughout the study, with an oscillation interval of approximately 2-3 years and a significantly higher seroprevalence in summer than in spring, supporting the oscillating infection dynamics hypothesized during a previous rabies study in a *Myotis myotis* colony. The maximum duration observed between successive positive serological statuses also demonstrated the potential persistence of neutralizing antibodies for at least 4 years. At last, juveniles and female adults seemed to act as distinct drivers of the rabies virus dynamics but their exact role still need to be specified.

VACCINATION AND INTRA-CAGE TRANSMISSION OF A RECOMBINANT PARAINFLUENZA VIRUS 5 EXPRESSING RABIES LYSSAVIRUS GLYCOPROTEIN IN THE BIG BROWN BAT (*EPTESICUS FUSCUS*)

James A. Ellison, Matthew R. Mauldin, Clint N. Morgan, Felix R. Jackson, William C. Carson, Huiling Wei, Kelsey Briggs, Nadia Gallardo-Romero, Biao He, Christina L. Hutson

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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, individuals of all species examined have tested positive for rabies. Over the last 20 years, the majority of human rabies cases in the US were identified as Rabies lyssavirus (RABV) variants typically detected in bats. Rabies is preventable by vaccination, and oral vaccination programs aimed at carnivores such as skunks, foxes, and raccoons have led to the elimination of rabies from large areas, and are effective in controlling rabies epizootics in terrestrial wildlife. Many species of bats are gregarious and live in large colonies, sometimes approaching thousands. A vaccine that is transmitted orally and/or nasally among individuals, and also capable of inducing immunity to rabies among and between bats within a large colony is clearly worth exploring. In this study we evaluate the efficacy of a novel recombinant canine parainfluenza type 5 rabies vaccine (PIV5-G) in bats against a lethal RABV challenge. In this study, seronegative big brown bats (n=24) were divided into three groups. Group 1 received PIV5-G vaccine (n=9), group 2 received a placebo (n=10) and were co-housed with group 1, group 3 (n=5) served as the challenge control and were housed in a separate laboratory until the day of the challenge (45 days post vaccination, pv). High mortality (80%) was observed among seronegative bats, and half of the PIV5-G vaccinated bats had detectable RABV neutralizing antibodies (rVNA) on day 13 pv. By day 30 pv, 40% of the placebo bats had detectable rVNA demonstrating intra-cage transmission of PIV5-G to con-specifics. Our results provide a contemporary example of herd immunity, and support the use of PIV5-G as a rabies vaccine in bats administered via the intranasal route.

PHYLOGEOGRAPHIC ANALYSIS OF RABIES IN MEXICO 2014-2016

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Paralytic bovine rabies exists only in Latin America. This is due, among other factors, to the presence of environmental and geographical conditions that define the presence and distribution of the vampire bat in some regions, which is the reservoir of the virus that causes the disease. On the other hand, genetic differences among different rabies virus strains and isolates have also been described. In this paper, the authors describe a strong and clear relationship between different viral lineages isolated in Mexico and their geographical origins, surprisingly reaching the municipal level. This implies that the habitats of the vampire bats are discontinuous, and well limited, and mainly, that the vampire bats don't travel beyond certain ecological barriers still to be determined for every municipality. In order to arrive to this conclusion, the authors analyzed the sequences of 345 positive rabies samples received at the OIE Rabies Reference Laboratory in CENASA / SENASICA, collected around the country by different institutions from 2014 to 2016. After sequencing, the results were analyzed at the Laboratory of Bioinformatics at the Escuela Superior de Medicina, Instituto Politécnico Nacional. In these three years, 19 Mexican states were affected by paralytic bovine rabies, mainly the state of Veracruz, in the Gulf of Mexico. Although there are national campaigns to control bat populations and bovine rabies in Latin America, this is still a problem. The definition of the vampire bat movements associated with the determination of the virus lineage should allow performing more efficient control measures of the vampire bat populations. During an outbreak, once phylogenetically characterized the lineage of an isolate, regional control measures can be taken only in the geographically limited region that is affected by every specific lineage, and not based on a ratio around the cases, avoiding over-reactions.



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DIAGNOSTICS/DIAGNÓSTICOS

ORAL PRESENTATIONS/PRÉSENTATIONS ORALES/PRESENTACIONES
ORALES/APRESENTAÇÕES ORAIS

MODERATORS/MODÉRATEURS/MODERADORES:

Dr. Susan Moore

Dr. Nidia Arechiga-Ceballos

SEROLOGICAL RESPONSE TO RABIES NUCLEOPROTEIN IN RECENTLY INFECTED VERSUS VACCINATED INDIVIDUALS USING AN N-PROTEIN ELISA

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Antibody detection is an important indicator of immune response after exposure to rabies virus (RABV) by either infection or vaccination. Human ante-mortem diagnosis relies on detection of binding antibodies by indirect immunofluorescence assay (IFA) and neutralizing antibodies by rapid fluorescent focus inhibition test (RFFIT) against RABV in serum or cerebrospinal fluid (CSF). These two assays detect different types of antibodies predominantly those directed against nucleoprotein (N) and glycoprotein (G), by IFA and RFFIT, respectively. While glycoprotein is more immunogenic and anti-G antibodies can neutralize RABV infection, nucleoprotein is the most abundantly expressed protein following RABV infection and hence would also elicit a robust immune response. However, antibody responses following rabies vaccination, which consists of inactivated RABV, may be more skewed towards immunogenic G, which is on the surface of viral particles compared to N, which is encapsulated in the viral particles. Hence, RFFIT is performed to determine post-vaccination and after infection neutralizing antibodies (anti-G), whereas IFA is performed only to assess response after infection. In addition, neutralizing antibodies are detected only in serum after vaccination compared to infection, where they can be detected in both serum and CSF. Thus, immune responses may be significantly different in infected versus vaccinated individuals. In this study, we have attempted to determine if there are differential responses using a nucleoprotein specific enzyme linked immunosorbent assay (ELISA) with a recombinant E. coli expressed purified rabies virus N protein in ante-mortem samples compared to vaccinated samples. The outcomes of the study and potential use of an N-protein ELISA for characterization of human ante-mortem samples will be presented.

EVALUATION OF DIFFERENT PARAMETERS FOR VIRUS ISOLATION IN N2A CELL CULTURE FOR RABV DIAGNOSIS IN HORSES

Danilo André Amanajás Maués Lemos; Karin Correa Scheffer, Keila Iamamoto, Karen Asano, Graciane Maria Medeiros Caporale, Adriana Candido Rodrigues, Francielle Cristina de Freitas, Enio Mori, Willian Oliveira Fahl

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For effective epidemiological surveillance of equine neurological diseases, laboratory diagnosis of rabies virus is necessary among encephalitis affecting equids. However, when compared with samples from other species, a lower sensitivity (76.6%) was observed in direct immunofluorescence for equine samples. The objective of this work was to evaluate different parameters used for viral isolation technique in N2a cell culture for equine central nervous system (CNS) samples in order to increase the technique sensitivity. For this purpose, 35 CNS samples from horses were selected, of which 25 of which were positive and 10 were negative for rabies. The modified parameters tested were: different concentrations of CNS suspensions, use of different types of diluents and consecutive passages. Suspensions were prepared in 5%, 10%, 20% and 30% concentrations with two types of diluents; the first using the PBS diluent (compounded of 0.85% buffered saline, added with 2% fetal bovine serum rabies antiviral antibodies free and 0.1% antibiotic) and the second one using Eagle's Minimum Essential Medium (MEM). In addition, 3 consecutive passages were performed for a possible increase of viral load. There was no difference in positivity with consecutive passages. It was verified that the 30% sample dilution resulted in loss of monolayer confluence, making the technique unfeasible. However, a dilution of 5 to 20% are recommended, since these concentrations showed a better result in positivity. A higher positivity was observed in the samples diluted in MEM, in concentrations 5% and 10% ($8/25 = 32\%$). Although it is recommended to prepare suspension for virus isolation with MEM, many laboratories use PBS diluent to reduce reagent costs. However, these results show that the replacement of the diluent type may lead to a lower diagnostic sensitivity, impairing the diagnosis by viral isolation in cell culture. Thus, these results show that the replacement of the diluent MEM may improve the diagnostic sensitivity, aiding in the diagnosis by viral isolation in cell culture.

DEVELOPMENT AND EVALUATION OF A METHOD FOR AUTOMATED DETERMINATION OF RABIES VIRUS NEUTRALIZING ANTIBODY TITERS BY THE FLUORESCENT ANTIBODY VIRUS NEUTRALIZATION TEST

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Animals traveling from rabies endemic areas to rabies-free areas require serological proof of adequate response to rabies vaccination, rabies virus neutralizing antibody (RVNA) titer of at least 0.5IU/mL. The Fluorescent Antibody Virus Neutralization (FAVN) test is an approved, and commonly used, assay for this purpose. The threshold of 0.5IU/mL is an objective measure of the level of protection afforded by circulating antibodies. This objective measure is affected by a degree of subjectivity due to the nature of the assay. The microscopic readings performed by human are inherently subjective; additionally this process is labor intensive. This study's objective was to reduce subjectivity, as well as save time and energy on this phase of the assay by development of an automated method for determining the RVNA level in serum. Preliminary studies using the BioTek Cytation and Gen5 software showed promise. 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 the ED50 and IU/mL of the sample. An optimal set of thresholds and settings was determined. The method presented shows good correlation between automatically generated data and manually generated data (R^2 ranged from 0.98 to 0.99). After performance of a full validation of the FAVN assay with automated readings, it is anticipated this method will increase throughput while maintaining or improving precision and accuracy.

DETECTION OF RABIES VIRUS IN CRANIAL LAVAGE OF NATURALLY INFECTED BATS - PARTIAL RESULTS

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Bats found in Brazil are usually small, so in some situations there are limitations in obtaining sufficient material to perform the various techniques used in the laboratory diagnosis of rabies. In these circumstances, the use of cranial lavage could be an alternative to this restriction. For this, the objective of this study was to evaluate the infectivity of rabies virus in samples of cranial lavage of positive bats by the viral isolation in cell culture (VICC). The bats carcasses were stored in a freezer at -20°C and were submitted at least to one freezing and thawing. A total of 74 animals diagnosed positive for rabies by at least one technique were selected, 26 of which animals were from the year 2015 and 48 from 2016. There were also randomly selected 10 animals that obtained a negative result. The skullcap was scraped with the aid of sterile tips and then 600 to 1000µL of diluent (0.85% buffered saline, added with 2% Bovine Fetal Serum, free of rabies antiviral antibodies and 0, 1% antibiotic) were added. All suspensions were added in N2a cell culture for viral isolation. From the total of 74 suspensions, 29 yielded positive by VICC, being 34.6% (9/26) of the year 2015 and 41.6% (20/48) of 2016. All 10 negative samples had their results confirmed by VICC. These results indicate that in 39.2% (29/74) of the studied samples, the virus remained infective, but the fact that the bats were previously manipulated, being frozen and thawed at least once, and were maintained at -20°C per a long period (1 or 2 years) may have caused considerable loss of infectivity. Therefore, it is suggested that molecular tests should be performed on these samples to evaluate the presence and the amount of virus.

DEVELOPMENT OF THE REAL-TIME POLYMERASE CHAIN REACTION TECHNIQUE FOR THE DIAGNOSIS OF RABIES

Yuliet Ramirez Cintra; Yanislet Cordero Aguilar; Yahisel Tejero Suarez; Oney Ortega Granda; Dailenys Perez Martinez; Maria de los Angeles Ribas Antunez

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Rabies is a lethal zoonotic viral caused by the rabies virus. The diagnosis in laboratories is necessary for the confirmation of the illness in animal and the preventions of human rabies. In Cuba the diagnosis is by immunofluorescence direct (IFD) assay, however, molecular tools are becoming more widely accepted and accessible for the diagnosis of rabies worldwide. It is expected that the use of molecular techniques improve the quality, accuracy and speed of rabies diagnosis, with the global vision of rabies elimination in developing countries. The objective of this study was to strengthen the diagnosis of rabies in Cuba with the introduction of molecular techniques as Real time PCR. To obtain a positive control, a fragment of the N gene was performed from sample 273/12, which was cloned into the pGEM-T vector, transformed into a strain of E. coli XL Blue and linearized using the restriction enzyme PstI. The standardization conditions of the real-time polymerase chain reaction (RT-PCR) technique were established and were evaluated in suspicious samples of the disease. The technique showed a sensitivity of 100% and a specificity of 91.7% when compared to IFD and 95.6% with PCR. A positive predictive value of 92.8% was obtained for the IFD and 96.5% for the RT-PCR and the negative predictive value was 100% for both techniques. The RT-PCR was evaluated in suspected rabies samples with excellent results, offering the possibility of having a technique with better sensitivity to detect samples with low viral load. The introduction of validated diagnostic tests capable of confirming the presence of rabies virus in clinical samples will undoubtedly be a fast, accurate and cost-effective way, leading to the combination of vaccination with the diagnosis for the human treatment of rabies and strengthening epidemiological and laboratory surveillance of rabies in Cuba.

IMPROVED RABIES DIAGNOSTICS BY ADDING THE PAN-LYSSAVIRUS REAL-TIME RT-PCR ASSAY LN34 TO THE STANDARDIZED PROTOCOL FOR RABIES TESTING

Gigante CM, Orciari L, Condori RE, Gao J, Wilkins K, Yager P, Wallace R, Reynolds M, Olson V, Li Y.

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During 2016, the pan-lyssavirus Taqman real-time RT-PCR assay LN34 was used for rabies diagnostics in parallel testing with the direct fluorescent antibody (DFA) tests in a large scale pilot program at 14 different domestic and international laboratories. The CDC rabies laboratory provided protocols, reagents, and technical support through email communications and conference calls. LN34 assay performance in each lab was monitored using a standardized artificial positive control. The diagnostic algorithms incorporated into the LN34 assay provided a standardized and objective rabies diagnostic process. The LN34 assay also includes a real-time RT-PCR assay measuring host mRNA level to determine sample quality and monitor errors in assay setup. The amplicon produced by the LN34 assay can be directly used for rapid sequence typing of positive rabies samples. In total, nearly 3000 rabies suspect animal and human clinical samples were tested. The LN34 assay did not produce any false negative results from verified samples. The diagnostic specificity and sensitivity of the LN34 assay are 99.1% and 99.3%, respectively. The discordant results between LN34 and DFA were due to the relatively high number of inconclusive results from the DFA test or samples with very low level of rabies virus RNA. The LN34 assay was able to improve the accuracy of rabies diagnostics by reducing the number of inconclusive DFA results, correcting over 10 false positive DFA results, and also identifying a false negative DFA result in a domestic laboratory. By adding the LN34 assay to the rabies diagnostic protocol, we can further improve the national standardized protocol for rabies testing by eliminating the elements causing non-specific reactions in current DFA tests and improve rabies diagnostic sensitivity.

LONG-TERM PRESERVATION OF INACTIVATED RABIES LYSSAVIRUS RNA

Smith Todd, Gigante Crystal, Li Yu

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Improved sampling process, storage and transportation of rabies samples are greatly important to improve rabies diagnostics and surveillance. Live rabies virus (RABV) represents a serious biosafety hazard. Finding alternative reagents that can inactivate the virus and stabilize the viral RNA at the point of sampling is necessary. In this study we evaluated the ability of DNA/RNA Shield buffer, paper based FTA card and RNA extraction reagent TRIzol to inactivate high titer ($>10^7$ ffu/ml) of laboratory strain RABV-ERA and their capacity to preserve RNA for long-term storage. As chemicals in those inactivation reagents are toxic to cell culture, we used Zeba spin desalting column to remove the cytotoxic components. In addition, we compared the stabilities of a brain suspension (1.8×10^5 ffu/ml) of raccoon RABV variant in TRIzol at two different temperatures. The RABV RNA was tested by Pan-Lyssavirus Taqman real time RT-PCR assay LN34, and the integrity of viral RNA was determined by using the traditional end point RT-PCR. All the reagents were highly toxic to cell culture at a 10^{-3} dilution. Zeba Spin desalting columns effectively removed the cytotoxicity of TRIzol and FTA Card reagents. However, cytotoxicity from DNA/RNA Shield was not completely removed. 2 Ct value of nonspecific binding of RABV to the Zeba column was observed based on the LN34 testing results. RABV suspension stored on FTA card deteriorated after 28 days at room temperature with a decrease of 10 Ct value of detectable RNA level, while RABV RNA in the reagent DNA/RNA Shield and TRIzol were much more stable with only a 2 Ct value decrease under the same conditions. DNA/RNA Shield and TRIzol can be used for RABV inactivation and long-term storage.

RABIES DIAGNOSIS IN THE USA - TIME FOR A REVISION TO THE NATIONAL STANDARD DFA PROTOCOL?

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



WILDLIFE RABIES/RAGE DE LA FAUNE/RABIA DE LA VIDA SILVESTRE/RAIVA DOS ANIMAIS SELVAGENS

ORAL PRESENTATIONS/PRÉSENTATIONS ORALES/PRESENTACIONES ORALES/APRESENTAÇÕES ORAIS

MODERATOR/MODÉRATEUR/MODERADOR:
Dr. Rich Chipman

TEN YEARS OF ORAL VACCINATION AND SEROLOGIC MONITORING OF RACCOONS: WHAT HAVE WE LEARNED SO FAR?

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Oral rabies vaccination (ORV) is the most widespread method used to control the spread of rabies in wildlife. The effectiveness of this approach is evaluated by characterising the prevalence of rabies virus-specific (RV) antibodies in targeted animals in vaccinated areas. However, most post-ORV studies show considerable variation in seroprevalence among years and vaccinated areas, suggesting the interplay of many factors that need to be considered to improve ORV campaigns. Our objective was to evaluate the influence of individual and populations characteristics of raccoons (*Procyon lotor*), environmental variables and baiting strategy on the probability of being seropositive for RV antibodies. To do this, we analysed the long-term database of post-ORV studies conducted in Québec, Canada. Between 2007 and 2016, 4805 individuals were captured in vaccinated areas located along an agricultural-forest gradient. For each individual, we determined sex, age class, and characteristics of baiting location (i.e. type (aerial vs. hand), bait density, frequency, and timing). We also quantified relative abundance of raccoons and landscape composition and structure within a 2 km radius around each capture site. Our results demonstrate high annual variability in herd immunity with seroprevalence ranging from 30% to 66% for a given trapping cell. Neither vaccine characteristics nor serologic method could explain the observed variations in seroprevalence. Potency tests on subsamples of vaccine baits showed that virus titers were adequate and stable over time indicating that the ONRAB® baits distributed were potent. The serologic method used (c-ELISA) was reliable as comparisons on a subsample of sera gave concordant results with virus neutralization tests. Analyses of the local and annual production of croplands in the vicinity of each raccoon were used to assess how habitat productivity could affect bait attractiveness and uptake, and thus, seroprevalence. Results will be discussed in the context of how future ORV campaigns can be improved.

RE-INCURSION OF RACCOON RABIES VARIANT INTO ONTARIO CANADA; AN URBAN CONTEXT

Tore Buchanan

Presenter: Tore Buchanan

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After the successful elimination of raccoon rabies variant from Ontario, Canada in 2005, a re-incursion of raccoon rabies variant occurred in Ontario in 2015. Rabies control knowledge, management techniques and technologies have changed considerably in the last 10 years. The specific circumstances in each outbreak are also different, particularly in regard to the rural versus urban context of the two outbreaks. We compare the current outbreak situation in Ontario to the previous outbreak and identify current and needed strategies for raccoon rabies variant management in an urban context.

WILDLIFE RABIES MANAGEMENT IN URBAN-SUBURBAN HABITATS - CHALLENGES AND INNOVATIONS

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Presenter: Jordona Kirby

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Managing rabies in terrestrial wildlife populations protects human and animal health and significantly reduces the economic impact of the disease. Since 1995, the Wildlife Services' National Rabies Management Program has worked cooperatively with local, state, and federal partners to manage rabies across large landscapes in a variety of habitats to prevent the spread of and ultimately eliminate specific terrestrial rabies variants. Wildlife rabies control in the U.S. is primarily achieved through distribution of oral rabies vaccine (ORV) baits by fixed or rotary-winged aircraft targeting raccoons, coyotes, foxes, and skunks in rural areas. In 2016, 5% (537,202) of the 11 million vaccine-baits distributed in the U.S. were distributed by hand (vehicle) and 1% (96,490) by bait stations in urban-suburban environments where aerial operations in fragmented and highly developed habitats is often not feasible. However, adequately baiting these strategically important habitats targeting raccoon and striped skunk populations is essential for working towards the goal of raccoon rabies elimination in the eastern U.S. Rabies management on the urban-suburban landscape is complex as a result of increased population densities and knowledge gaps in understanding the ecology of target species, patchy distribution, anthropogenic food sources, and non-target species bait competition, resulting in lower seroconversion rates and persistence of rabies cases compared to more rural environments. Innovative approaches to improve vaccination effectiveness and efficiency in areas traditionally hand baited are required to achieve success. Recent innovations include increased use of helicopter distribution, use of spatial technology including Point-of-Interest GPS and ArcGIS applications (Delaunay Triangulation) to refine existing baiting tactics, evaluation of bait station prototypes and densities, and a comprehensive spatial ecology study of raccoons and striped skunks in Burlington, VT relative to ORV hand bait distribution strategies. These collaborative efforts will ultimately improve wildlife rabies management in urban-suburban environments.

RABIES VIRUS ANTIBODIES FROM ORAL VACCINATION AS A CORRELATE OF PROTECTION AGAINST LETHAL INFECTION IN WILDLIFE

Susan M. Moore, Amy Gilbert, Ad Vos, Conrad M. Freuling, Christine Ellis, Jeannette Kliemt, and Thomas Müller

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Presenter: Susan Moore

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Both cell-mediated and humoral immune effectors are important in combating rabies infection, although the humoral response receives greater attention regarding rabies prevention. The principle of preventive vaccination has been adopted for strategies of oral rabies vaccination (ORV) of wildlife reservoir populations for decades to control circulation of rabies virus in free-ranging hosts. There remains much debate about the levels of rabies antibodies (and the assays to measure them) that confer resistance to rabies virus. In this presentation, data from published literature and our own unpublished animal studies on the induction of rabies binding and neutralizing antibodies following oral immunization of animals with live attenuated or recombinant rabies vaccines are examined as correlates of protection against lethal rabies infection in captive challenge settings. Analysis of our studies suggests that, though serum neutralization test results are expected to reflect in vivo protection, the blocking enzyme linked immunosorbent assay (ELISA) result at Day 28 was a better predictor of survival. ELISA kits may have an advantage of greater precision and ability to compare results among different studies and laboratories based on the inherent standardization of the kit format. We synthesize current knowledge and results of this study to guide meaningful interpretation of serology results for oral baiting monitoring programs.

A SPATIALLY-EXPLICIT SIMULATION MODEL OF MONGOOSE RABIES IN PUERTO RICO: INFLUENCE OF MONGOOSE ECOLOGY AND LANDSCAPE FEATURES

Caroline C. Sauvé, Aaron M. Anderson, Are R. Berentsen, Amy T. Gilbert, Erin E. Rees, and Patrick A. Leighton

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The small Indian mongoose (*Herpestes auropunctatus*) is a small carnivore introduced to numerous sugarcane growing islands to limit crop damage caused by rodents. In the Caribbean, this species represents a significant public health concern as the primary wildlife reservoir for canine rabies on several islands. This study adapted an existing individual-based, spatially-explicit simulation model, the Ontario Rabies Model (ORM), to explore the impact of mongoose ecology and landscape features on the dynamics and persistence of rabies in Puerto Rico where mongoose rabies is endemic. Model scenarios were used to assess the influence of five parameters associated with mongoose ecology for which there is limited information in the literature: elevation as a barrier to mongoose movement, dispersal distance of animals, habitat-associated differences in population density, contact rates among individuals, and level of rabies-induced mortality. Rabies persistence, prevalence and spatial distribution of infected animals after 150 years of simulation were compared among scenarios. Population density and contact rates among individuals were the most influential variables. A 25% reduction in density resulted in a 20% and 23% decrease in rabies prevalence and area affected, respectively. Similarly, decreasing contact rates among individuals by 50% lead to a 21% and 18% reduction in prevalence and area affected, respectively. Allowing 25% of animals to disperse further than 3 km annually increased both prevalence and area affected by 7%. Finally, suppressing the elevation-related resistance to movement increased rabies prevalence by 17%. Rabies-induced mortality rates were crucial for rabies persistence: both low and high mortality caused outbreaks to die out because of rapid population-level gains in natural immunity and of limited infectious population size, respectively. This study demonstrates the feasibility and utility of simulating mongoose rabies dynamics and provides insights into priority areas for further research to improve our understanding and management of mongoose rabies in the Caribbean.

EFFICACY OF ONRAB VACCINE BAIT DELIVERY AGAINST RABIES CHALLENGE IN RACCOONS

Amy Gilbert, Shylo Johnson, Nikki Walker, Chad Wickham, Alex Beath and Kurt VerCauteren

Presenter: Amy Gilbert

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In the USA, rabies virus (RABV) only circulates in wildlife species and the most significant wildlife reservoir from a public and agricultural health perspective is the raccoon (*Procyon lotor*). Management of rabies in wildlife reservoirs in the USA has relied principally on oral rabies vaccination (ORV) strategies using vaccine-laden bait delivery to free-ranging target hosts, in order to reduce the susceptible population to prevent spread of or eliminate RABV circulation. The objective of this study was to evaluate the efficacy of ONRAB vaccine baits against a lethal RABV challenge in captive raccoons. Sham or live vaccine baits were offered to 50 raccoons, and efficacy was evaluated in 46 raccoons, split into two trials of 17 and 29 animals respectively. Raccoons were challenged with a lethal dose of RABV 180 days post-vaccination, and observed for 90 days post-infection. Bait interaction scores varied across the two trials, and higher interaction scores were observed in the fall compared to spring trial. Although animal age was not a factor explaining variation in bait interaction scores, greater immunogenicity of the vaccine was observed in juvenile compared to adult raccoons and the geometric mean rabies virus neutralizing antibody titer among juvenile animals was higher than adults at all time points pre and post infection. The efficacy of ONRAB bait delivery was 85% (29 of 34) overall, ranging from 73% (8/11) to 91% (21/23) across the two trials. All sham-vaccinated raccoons (12/12) succumbed to rabies infection. These data indicate that ONRAB bait vaccination is efficacious in protecting adult and juvenile raccoons against rabies infection for a minimum of six months. These data complement experimental field trials in the evaluation of the ONRAB product for control of rabies circulation in free-ranging raccoon populations in the USA.

EVALUATION OF ORAL RABIES VACCINE BAIT UPTAKE BY SMALL INDIAN MONGOOSES: PRELIMINARY RESULTS FROM THE LAB AND FIELD

Are R. Berentsen, Kathleen M. Nelson, Richard B. Chipman, Ad Vos, Steffen Ortmann, Ken Gruver, Frank Boyed, Robert T. Sugihara, Cynthia G. Payne, Steven Volker and Amy T. Gilbert

Presenter: Are Berentsen

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The small Indian mongoose (*Herpestes auro-punctatus*) is a rabies reservoir in Puerto Rico and certain other Caribbean islands, but no effective control program targeting this host exists. We evaluated flavor preference and bait uptake of oral rabies vaccine baits by free-ranging mongooses. We established three 0.25 km² plots each containing transects alternating egg and unflavored baits. Each plot was evaluated once during the spring (dry season) and fall (wet season). Baits were monitored for up to five days or until at least 50% of baits were removed or rendered unavailable. Overall, 46% of all baits were removed. Sixty-one percent of egg-flavored baits were removed, vs. 32% of unflavored baits, although we documented both mongooses and rats removing comparable proportions of baits. We also evaluated iophenoxic acid (IPA) as a biomarker in captive mongooses, and report that 2.8 mg of methyl and ethyl-IPA provided adequate marking ability for 28 and 56 days, respectively. We conducted field trials at three 4 km² sites in SW Puerto Rico in the fall of 2016 and spring 2017. We hand distributed egg-flavored baits containing 2.8 mg ethyl (fall) and methyl (spring) IPA at 200 baits/km². We captured, tagged, and sampled mongooses for ten consecutive days following bait application for blood collection. Blood samples were analyzed for IPA residue by high performance liquid chromatography. During the fall trial, 88 unique mongooses were sampled, 55 (63%) had detectible levels of ethyl-IPA in their sera. Results from the spring trial are pending. The relatively high proportions of free-ranging mongooses marked during the field trials suggest promising bait uptake at the application rate evaluated. Special thanks to E. Bruce, C. Ellis, M. Grady, J. Humphrey, S. Johnson, J. Reynolds, M. Rivera-Rodriguez, H. Stockdale, C. Thomas, F. Torres-Toledo, C. Wickham and C. Zimmerman for exceptional field assistance.

KUDU RABIES IN NAMIBIA – RECENT EXPERIMENTAL STUDIES ON TRANSMISSION AND VACCINATION

Rainer Hassel, Ad Vos, Conrad Freuling, Steffen Ortmann, Peter Clausen, Siegfried Khaiseb, Juliet Kabajani, Jolandie van der Westhuizen, Mark Jago, Floris Bruwer, Thomas Müller

Presenter: Thomas Müller

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Rabies in the Greater Kudu (*Tragelaphus strepsiceros*) is unique to Namibia because it has not been reported in other countries where these antelopes occur, nor does it affect other game species in southern Africa to the same extent. Kudu rabies remains a major problem for Namibia as economy relies on kudu through the means of trophy hunting, game meat hunting and eco-tourism. Tens of thousands of Kudu have been claimed to succumb to rabies during two major epizootic outbreaks in the past four decades. Recently, rabies has again spread over a large portion of the kudu habitat with severe outbreaks having also been reported from the south of Namibia. Questions as to whether Kudus are special in rabies epidemiology (horizontal transmission) and whether there is an effective and practical way of vaccination still remain unanswered at best. In a proof-of-concept experimental study we focused on unravelling the conundrum of kudu rabies epidemiology and investigating the possibility of oral vaccination of kudus against rabies. Wild-caught kudu infected with a species specific rabies virus strain showed dose dependent incubation periods ranging from 12-15 (high dose) and 93-247 (low dose) days. While parenteral vaccination using inactivated rabies vaccines proved very efficient in kudu, it seems that they are rather refractory to the oral route of vaccine administration. Further studies need to be initiated to optimize oral vaccine delivery. Several candidate baits have been identified that were readily taken up by captive and free-living kudus. Finally, a bait distribution system has been suggested that maximizes uptake by the target and meanwhile minimizing uptake by non-target species. Problems with performance of standard serological assays underscore the need of validation for this particular species.

FIRST REPORT OF CONEPATUS MESOLECUS AND MEPHITIS MACROURA AS RABIES VIRUS RESERVOIRS IN NUEVO LEÓN STATE, MEXICO

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Rabies surveillance in the North America reported in 2015, 1.365 rabid skunks in the United States and 7 in Canada. In both countries, the species most commonly positive to rabies is *Mephitis mephitis*. In Mexico three antigenic variants circulate in skunks: AgV1 AgV8 and AgV10. AgV1 has a canid origin, while this variant had been eliminated in dogs' populations by means of vaccination campaigns, nowadays skunks' species in Chihuahua, Durango, Sinaloa and Sonora harbor this variant. AgV8 has been frequently associated with *Spilogale putorius* in: San Luis Potosi (SLP), Aguascalientes, Jalisco and Zacatecas. However, in the state of SLP, this variant has also been described in *Conepatus leuconotus*. Epidemiological studies performed by Velasco-Villa et al., 2002 and Loza Rubio et al., 2012 mentioned *S. putorius* as the main reservoir for rabies in skunks. However, other species such as *C. leuconotus* and *Spilogale* spp. with broader distribution in Mexico have been documented as reservoir of AgV8. AgV10 is restricted to skunks from Baja California Sur, although the reservoir species has not been correctly identified. Therefore, studies of rabies skunks in Mexico have been insufficient, especially when trying to identify species with a history of aggression or transmission to humans. The aim of this study was to identify the species of skunks and the lineage of rabies virus circulating in the Nuevo Leon State. The viruses were isolated from 2 skunks with human aggression history. Taxonomic identification proved that the species were: *Mephitis macroura* and *Conepatus mesoleucus*. Fluorescent antigen (FAT), antigenic characterization, RT-PCR and nucleotide sequencing were performed. Virus isolates were characterized as AgV8. This is the first report in Mexico that demonstrates the circulation of rabies virus in both species.



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INTERNATIONAL CONFERENCE
ON RABIES IN THE AMERICAS

RABIES CONTROL/CONTRÔLE DE LA RAGE/CONTROL DE LA RABIA/CONTROL DA RAIVA

ORAL PRESENTATIONS/PRÉSENTATIONS ORALES/PRESENTACIONES
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Mr. Tore Buchanan
Dr. Veronica Gutierrez

EARLY CONTROL RESPONSE TO THE RABIES OUTBREAK IN SARAWAK, MALAYSIA

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Sarawak, Malaysia announced a rabies outbreak on July 1st 2017 following the positive diagnosis of rabies in two children from the district of Serian. An emergency management disease committee was activated (Rabies Secretariat) incorporating all stakeholder groups including the Sarawak SPCA; a local NGO operating in Kuching that had conducted a rabies contingency workshop last year. Sarawak had been free of rabies since 1999. Active surveillance along the Kalimantan border of canine samples had been negative until 2015. Initial brain samples of dogs and cats from the Serian area were all positive using DFAT carried out in the veterinary diagnostic laboratory in Kuala Lumpur. The Department of Veterinary Services (DVS) comes under the lead government department for the outbreak that is Agriculture. DVS comprises of seven livestock vets and other staff covering the whole state. Small animal private veterinarians

service the city of Kuching's 800,000 people and their pets. Knowledge of rabies and controlling this disease was limited though different sectors had experience with other disease outbreaks in Sarawak and used strategies from these outbreaks in the initial response. Determining the extent of the outbreak has been difficult given most resources focused on mass dog vaccination of owned dogs to create buffers zones and rapid response to suspect rabid animals reported from hot-line and animal bite tracing. All front-liner personnel needed pre-exposure prophylaxis rabies (Verorab®) courses. Four dog bite assessment and treatment centres were established in Kuching and Serian. An Inter-government meeting with Indonesia had occurred given the molecular virus typing indicated the same virus was circulating in Kalimantan where thirteen human rabies deaths had occurred this year. Sarawak will need assistance to support continuation of vaccination campaigns, estimate dog population vaccinated percentage, develop local laboratory diagnostic capacity and to enhance surveillance.

RABIES CONTROL IN MANITOBA, CANADA: A ONE HEALTH APPROACH

Judy Hodge, Colleen Dudar, Richard Rusk, Richard Baydack

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Issue – In April 2014, the federal government transferred the management of rabies to the provinces and territories in Canada. In Manitoba, human, animal, and environment health impacts from infectious diseases were managed by separate government departments. Increased understanding of emerging and re-emerging infectious diseases has led to a broader acceptance and adoption of the ‘One Health’ concept, i.e. inter-disciplinary collaboration between experts in public, animal, and environment health. The Provincial Rabies Management Program was developed within a One Health-structured framework with the operation component being Manitoba Rabies Central (MRC), representing the departments of Manitoba Health, Seniors and Active Living (MHSAL); Manitoba Agriculture (MB Ag); and Sustainable Development (SD).

Program – MHSAL manages potential human exposures to rabies, including conducting risk assessments (RAs) and administering post-exposure prophylaxis; MB Ag manages potential domestic animal exposures to rabies, including RAs and quarantines, as well as sample collectors; and, SD provides expert advice on the sylvatic rabies cycle. Their staff participate as collectors to provide coverage in remote communities. Reports of potential rabies exposures to either humans or domestic animals are referred to regional public health or MB Ag, respectively. Sample pick-up requests from either regional public health or MB Ag are sent to MRC, who manages the database of sample pick-up requests. The database generates a unique sample reference ID number. The request is sent to MB Ag, who coordinates sample pickup, packaging, and shipping. Test results are received electronically at MRC and the relevant region for public health follow-up.

Outcome – MRC has seen a significant reduction in the number of samples tested while the number of positive samples has remained constant due to a more rigorous RA process, especially for potential human exposure. Having human healthcare providers conduct RAs directly allows for a more scientific RA, helping to mitigate emotive testing.

TARGETED RESPONSE TO PERSISTENT RACCOON VARIANT RABIES IN A RABIES CONTROL ZONE IN SOUTHWESTERN NEW BRUNSWICK

Michael R. Allan, James P. Goltz, Tony Cole, Paul Turmel, Joan Bourque, Elbridge Carr, Justin Carr, Kathy Cromwell, Vicky Birt

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On February 8, 2017, a striped skunk, embedded with porcupine quills and wandering around during daylight hours in the small village of Waweig, NB, was live-trapped in an abandoned barn and humanely euthanized. Testing confirmed that the skunk was rabid and infected with the raccoon rabies variant. Four rabid raccoons had previously been detected in this same general area in 2015, prompting aerial distribution of ONRAB oral rabies vaccine baits in 2015 and 2016. In response to the rabid skunk incident, the provincial government conducted a localized population reduction and surveillance effort using live traps in abandoned barns and buildings to remove any raccoons, skunks or foxes that may be incubating or infected with the rabies virus within 4 km of the primary case. Eight raccoons, four skunks and two porcupines were captured and humanely euthanized between February 17 and 24, 2017, utilizing a trapping effort of 310 trap nights. Three more raccoons and one more skunk were captured between March 6 and May 1, 2017, utilizing a trapping effort of 243 trap nights. A total of 11 raccoons, 5 skunks and 2 porcupines from this area were tested for rabies using the direct Rapid Immunohistochemistry Test. Two additional skunks, both from the same barn as the primary case, tested positive for rabies. On April 13, 2017, ONRAB oral rabies vaccine baits were distributed within 4 km of the primary case at a density of 140 baits/km². No additional cases of raccoon variant rabies have been detected in that geographic area or elsewhere in the province. Local residents have been assisting with surveillance efforts.

EVALUATION OF A TRADITIONAL MASS DOG VACCINATION CAMPAIGN SUPPLEMENTED WITH SPBNGAS-GAS ORAL RABIES VACCINE, HAITI, 2016

Todd G. Smith, Ad Vos, Franso Acky Fracciterne, Kelly Crowdis, Cornelius Chirodea, Alexandra Medley, Richard Chipman, Max Millien, Yunlong Qin, Jesse Blanton, and Ryan Wallace

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Dogs that are inaccessible for parenteral vaccination represent a major hurdle to eliminating canine rabies in low-resource settings. Alternatives such as oral rabies vaccine (ORV) for dogs should be considered. In Croix-des-Bouquets, Haiti in 2016 a traditional mass dog vaccination campaign was supplemented with ORV using a handout method. Boiled beef intestines served as the bait for a blister containing the rabies vaccine strain SPBNGAS-GAS. Of 590 dogs that received ORV, dogs' location, bait acceptance, vaccine exposure, and blister recovery were recorded by vaccinators for 291 dogs (49.3%). 107 dogs were enrolled in a sero-survey to determine vaccine efficacy. 78 dogs (72.9%) had successful follow-up blood draw 17 days post-vaccination. Various factors limited the time to follow-up and ability to relocate dogs. Seroconversion among orally vaccinated dogs was 59-78% consistent with previously reported ORV seroconversion in dogs but was significantly less compared to parenterally vaccinated dogs ($p < 0.05$). The beef intestine bait was well accepted and effective for ORV delivery to dogs in Haiti. The handout model used for this study resulted in very few unused ORV blisters left in the community decreasing the risk of non-target animal and human exposure. Consumption of the blister had no significant effect on seroconversion compared to puncturing and expectorating the blister. The seroconversion rate among dogs that received ORV indicates SPBNGAS-GAS vaccine could be used effectively in Haiti to improve vaccination coverage among inaccessible dogs where parenteral vaccination has failed to reach desired coverage levels.

WHAT IS THE RISK? A PROBABILISTIC SAFETY MODEL TO PREDICT HUMAN ADVERSE EVENTS TO MODIFIED LIVE ORAL RABIES VACCINES FOR DOGS

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Parenteral vaccination, provided at central point vaccination clinics, has long-been the cornerstone of canine rabies elimination programs. Many developed countries have eliminated canine rabies by utilizing this methodology. However, few low and middle income countries achieved canine rabies elimination. A study in 2017 found that countries free of canine rabies had significantly higher levels of infrastructure when compared to enzootic countries. Limited infrastructural capacity may result in reduced efficacy of traditional vaccination methods (ie parenteral vaccination through central point campaigns). Furthermore, inaccessible dogs may be more common in less developed countries, which may negatively impact participation at central point vaccination clinics. When traditional methods of vaccination have not achieved the desired impact, alternative methods should be considered. Oral vaccines have been used for over 20 years in Europe and North America to control rabies in wildlife species, however, due to concerns about human exposure to modified live rabies vaccines, oral vaccination of dogs has not been widely accepted. CDC and partners developed a probabilistic model to identify pathways in which a person may become exposed to an oral rabies vaccine, and the predicted health outcomes from the exposure. This model was utilized to assess the modified live oral rabies vaccine SPBN GASGAS. Literature searches were conducted to fit parameter values; missing values were obtained from unpublished data provided by the vaccine manufacturer. Mean values and ranges were input into the program TreeAge, which conducted a Monte-Carlo permutation simulation and provided an expected rate of adverse events to humans. When utilizing the SPBN GASGAS product in which unconsumed bait and bait remnants are immediately retrieved, the model predicts three vaccine-associated human deaths for every 1 billion baits distributed. This model can be used to support the further inclusion of safe and effective oral vaccines into routine dog vaccination programs.

CAPACITY BUILDING FOR GLOBAL RABIES ELIMINATION

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Presenter: Louise Taylor

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Over the last decade, international rabies experts and stakeholders have been developing mechanisms, tools and training to support countries as they move forward with rabies control efforts. There are now many examples of how such initiatives have had an impact in small scale efforts in countries across Africa and Asia.

Now, with a global goal of an end to human rabies mediated by dogs set for 2030 we need a concerted plan and effort to apply this expertise and tools uniformly to support countries as they move towards regional and the global goals for canine rabies elimination.

A clear strategy has been developed by the Global Alliance for Rabies Control (GARC) and other partners whereby regional rabies meetings introduce countries to tools such as the Canine Rabies Blueprint and the Stepwise Approach toward Rabies Elimination. This allows countries to design an appropriate elimination strategy for their setting and identify priority areas for capacity building. Appropriate elements of a standardized set of international training methods and tools can then be utilized to strengthen health systems as necessary to be able to execute elimination strategies. Vaccine banks and stockpiles can enable countries to access quality vaccines at reasonable prices. Regional meetings facilitate data collection into powerful and locally adaptable regional databases and allow countries to support each other as they learn from their experiences.

Building on the 2015 Rationale for Investment and the 2016 Global Framework for the elimination of human rabies transmitted by dogs, the tripartite organisations and GARC are developing a Global Rabies Business Plan. This initiative seeks to fund capacity building interventions in countries, raise the disease's profile at the global level, monitor progress and attract more resources to enable significant strides towards elimination of canine rabies to be made in Africa and Asia where its impacts are greatest.

DEMOGRAPHIC AND ECOLOGICAL SURVEY OF DOG POPULATION IN ABA, ABIA STATE, NIGERIA

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Presenter: Gbeminiyi Richard Otolorin

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Dog ecology is essential in understanding the distribution, structure, population density of dogs and pattern of dog ownership in any given area. A cross sectional study was designed to study dog ecology in Aba, Abia state Nigeria from April to June 2013. The study revealed that the 500 households surveyed possessed 5,823 individuals and 747 dogs, giving a dog to human ratio of 1:7.8 with an estimated 68,121 number of dogs in Aba, Abia state Nigeria. About 495/747 (66.3%) of the dogs were exotic and 465/747 (62.2%) were males. A total of 319/500 (63.8%) of the household had fences that restrained dog movement and there was no incidence of dog bite in 447/500 (89.4%) of the households surveyed. There were associations between breeds of dogs ($\chi^2 = 79.8$, $df = 2$, $P < 0.005$), age of dogs ($\chi^2 = 22.9$, $df = 2$, $P < 0.005$) and vaccination against anti-rabies. Exotic breed (adjusted OR = 0.39; CI = 0.23- 0.65) and local breed of dogs (adjusted OR = 0.08; CI = 0.04 – 0.14) had less odds of being vaccinated as compared to cross breed of dogs. About 126 dogs (2.5 dogs per street) was estimated from street counts survey. The relative high dog to human ratio and low vaccination coverage of owned dogs population pose public health concerns requiring adequate public health education and proper anti-rabies vaccination coverage of dogs in the study area.

IN-DEPTH ANALYSIS OF SPECIES DIFFERENCES IN VACCINE UPTAKE EFFICIENCY – FOX AND SKUNK AS EXAMPLES

Verena te Kamp, Ad Vos, Conrad M. Freuling, Boris Hundt, Christiane Kaiser, Sabine Nemitz, Andreas Neubert, Peter Schuster, Reiner Ulrich, Jan Schinköthe, Stefan Finke, Thomas Müller

Presenter: Conrad Freuling

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In oral vaccination using attenuated and recombinant rabies vaccines clear differences have been observed in vaccine titers needed to induce a protective immune response against rabies in different reservoir species. In a proof-of-concept, it was shown that the immunogenicity of the vaccine virus strain, SPBN GASGAS, in the red fox and in striped skunk differed and correlated with virus dissemination in the oral cavity. In order to further investigate the replication of attenuated RABV after oral administration, foxes and skunks were orally vaccinated with a recombinant, GFP expressing RABV with a genetic background of the oral vaccine strain SAD L16. By confocal laser scan imaging and immunohistochemistry of various tissue samples, palatine tonsils were confirmed as a main site of virus replication with characteristic and locally restricted distribution of infected cells in foxes only, while infected cells were not detected in palatine tonsils of skunks. RT-qPCR screening of oropharyngeal tract tissues supported that the skunk as a rather refractory species for oral immunization are less efficiently infected. Notably, also the time course of infection was restricted, indicating that the main triggers for an induction of the adaptive immune response arise in the first four days after oral vaccination. Our data support that carnivore reservoir species substantially differ in their susceptibility to oral live vaccine infection and that this may determine potency of an oral RABV live vaccine.

FACTORES DE LA POBLACION CANINA Y FELINA QUE SE RELACIONAN CON LA PRESENTACION DE RABIA EN EL ESTADO DE TLAXCALA, MEXICO

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La RABIA se ha caracterizado como un importante problema de salud en México, en estos últimos años ha sido encarada en su justa dimensión asignándole alta prioridad y proporcionándole recursos crecientes. Tlaxcala se localiza en la región centro de la República Mexicana, Ancestralmente junto con otras entidades de la región formaron un corredor endémico para la rabia; está dividida en 60 municipios debido a la gran diversidad de costumbres entre la población, el cual el perro representa una figura importante ya que además de hacerle compañía a su dueño durante sus labores, es también quien resguarda la seguridad de sus domicilios. El presente trabajo se llevó a cabo en 16 localidades seleccionadas aleatoriamente (3 Localidades Rurales, 8 Semiurbanas y 5 Urbanas); lo que permite tener un panorama de la realidad de todo el Estado. El objetivo fue conocer los factores de la población canina y felina que se relacionan con la presentación de rabia en el Estado, para ello se analizaron las siguientes variables: Especie, Edad, Sexo, Cobertura de vacunación antirrábica, Cobertura de Esterilización, Procedencia de los animales, Número de gestaciones, Cachorros por camada, Alimentación cotidiana, Número de viviendas con animales, y Personas agredidas que acudieron a recibir atención Médico antirrábica

Estudio transversal

La información se recolecto mediante un cuestionario diseñado específicamente para esta actividad, concentrado en un dispositivo móvil utilizando códigos QR. La información se concentra en una plataforma de manera automática y puede ser consultada en tiempo real. La actividad se desarrolló en el 100% de viviendas de cada una de las localidades.

Los resultados obtenidos pueden contribuir al mejoramiento y la reorientación de las actividades del programa de prevención y control de la rabia, con el fin de limitar el riesgo de presentación de casos de rabia.



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**EPIDEMIOLOGY AND SURVEILLANCE/ÉPIDÉMIOLOGIE ET
SURVEILLANCE/EPIDEMIOLOGÍA Y
VIGILANCIA/EPIDEMIOLOGIA E VIGILÂNCIA**

**ORAL PRESENTATIONS/PRÉSENTATIONS ORALES/PRESENTACIONES
ORALES/APRESENTAÇÕES ORAIS**

MODERATORS/MODÉRATEURS/MODERADORES:

Dr. Joanne Tataryn

Dr. Ivanete Kotait

EVALUATING THE ENDEMIC DOG VACCINATION RATE ON THE NAVAJO NATION AND POTENTIAL RISK TO THE PUBLIC

Scott C. Bender, David L. Bergman

Presenter: Scott Bender

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The WHO recommends that a minimum of 70% of dogs in a population should be immunized to eliminate outbreaks of rabies, based upon years of rabies vaccine campaigns and the mathematics of mass vaccination ($q_c = 1 - 1/R_0$). Starting in 2001, the Navajo Nation and USDA- Wildlife Services have conducted cooperative study projects with the intention of finding a viable baits and oral rabies vaccines (ORV) that could be used for rabies vaccinations in areas with large free roaming dog populations. Because the target dog class has been free roaming dogs, rabies titers were evaluated in a majority of these studies from dogs that were collected from free roaming dog populations impounded by the Navajo Nation animal control program. In an effort to better determine the current herd immunity level of the Navajo Nation free roaming dog population, we conducted a retrospective review of multiple studies, recording those evaluated for pre-study rabies titer levels. Based upon the review, the Navajo Nation dog population is not only well below the 70% titer rate recommended for eradication, it is also well below the static 59% titer rate for dogs that should be vaccinated at any one time to control or prevent rabies outbreaks.

RABIES SURVEILLANCE IN THE UNITED STATES DURING 2016

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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 comprises > 130 state public health, agriculture, and university laboratories performing the standard direct fluorescent antibody test. In addition, the USDA Wildlife Services submitted active surveillance data on animals tested with the direct rapid immunohistochemical test, accounting for about 5% of all animals submitted for rabies testing. Data submitted by states, territories, and the USDA Wildlife Services 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 2016. 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. This report will provide a summary of human rabies cases from January 2003 through September 2017.

USE OF THE DIRECT RAPID IMMUNOHISTOCHEMICAL TEST (DRIT) IN A LARGE SCALE SURVEILLANCE PROGRAM TO MONITOR THE DISTRIBUTION AND SPREAD OF RABIES IN WILDLIFE

Kevin Middel, Christine Fehlner-Gardiner, Natalie Pulham and Tore Buchanan

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Following an incursion of the mid-Atlantic raccoon variant of rabies virus into southern Ontario, Canada in late 2015, the direct Rapid Immunohistochemical Test for rabies (dRIT) was employed on a large scale to establish the outbreak perimeter and to diagnose specific cases to inform rabies control management actions. Following detection of the first positive case in 2015, our large scale enhanced surveillance program has tested over 7,000 wildlife carcasses with over 95% of all positive cases initially diagnosed through the dRIT procedure. When compared with the gold standard Fluorescent Antibody Test (FAT) the dRIT was found to have a sensitivity of 100% and a specificity of 98.2%. Positive and negative test agreement was shown to be 98.3% and 99.1%, respectively, with an overall test agreement of 98.8%. The average cost to test a sample was \$3.13 CAD for materials and hands-on technical time to complete the test is estimated at 0.55 h. The ability to perform rapid, inexpensive testing procedures for rabies enables more responsive control strategies, near-real time tracking of spread and assists with decisions regarding targeted wildlife surveillance. The dRIT procedure has been found to be accurate, fast, inexpensive, easy to learn and perform, and an excellent tool for monitoring the progression of a wildlife rabies incursion.

RISK FACTORS FOR INADEQUATE ANTIBODY RESPONSE TO PRIMARY RABIES VACCINATION IN DOGS UNDER ONE YEAR OF AGE

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Ensuring the adequacy of response to rabies vaccination in dogs is important, particularly in the context of pet travel. Few studies have examined the factors associated with dogs' failure to achieve an adequate antibody titer after vaccination (0.5 IU/ml). This study evaluated rabies antibody titers in dogs after primary vaccination. Dogs under one year of age whose serum was submitted to a reference laboratory for routine diagnostics, and which had no prior documented history of vaccination were enrolled (n = 8,011). Geometric mean titers (GMT) were calculated and univariate analysis was performed to assess factors associated with failure to achieve 0.5 IU/mL. Dogs vaccinated at >16 weeks of age had a significantly higher GMT compared to dogs vaccinated at a younger age (1.64 IU/ml, 1.57 – 1.72, ANOVA p < 0.01). There was no statistical difference in GMT between dogs vaccinated <12 weeks and dogs vaccinated 12-16 weeks (1.22 IU/ml and 1.21 IU/ml). The majority of dogs failed to reach an adequate titer within the first 3 days of primary vaccination; failure rates were also high if the interval from vaccination to titer check was greater than 90 days. Over 90% of dogs that failed primary vaccination were able to achieve adequate titers after booster vaccination. The ideal timing for blood draw is 8 – 30 days after primary vaccination. In the event of a failure, most dogs will achieve an adequate serologic response upon a repeat titer (in the absence of booster vaccination). Booster vaccination after failure provided the highest probability of an acceptable titer.

LESSONS FROM VIRTUAL LANDSCAPES: SPACE-TIME CHARACTERISTICS OF RABIES SPREAD

Erin E. Rees, Bruce A. Pond, Rowland R. Tinline, Erica Newton, Kevin Middel, Denise Bélanger

Presenter: Erin Rees

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In previous studies using the Ontario Rabies simulation model we have found that vaccination strategy and level of host habitat heterogeneity can interact to influence rabies dynamics, and in some cases, be counter-productive to rabies control. Our current project assesses the space-time characteristics of rabies spread to understand how vaccination and habitat heterogeneity influence the location, direction and speed of rabies spread.

We examine the impact of uniformly applied vaccine bait treatments across landscapes of varying habitat heterogeneity on rabies spread during epizootic and enzootic periods using the simulation model. We use three virtual landscapes of the same overall habitat quality: one is homogeneous of medium quality habitat; another has randomly arranged small patches of high and low quality habitat and the third has thin corridors of high quality habitat surrounded by large patches of low quality habitat. We measure spread along chains of infection between rabid individuals with metrics such as movement speed, step length and displacement.

Results show that rabies spreads faster during epizootics than enzootics. As the epizootic ages, chains more frequently “wander” across the landscape. Increasing vaccination slows spread by reducing step length and chains are more likely to follow corridors of high quality habitat which have more remaining susceptible hosts. The impact of vaccination to slow spread is far reduced during the endemic phase. Our results suggest rabies detection is more likely when surveillance is targeted to patches of high quality habitat in both epizootic and endemic phases. Furthermore, increasing vaccination effort has a dwindling impact to control spread as the epizootic ages into the enzootic phase.

MONITORING AND TRACKING RABIES IN THE UNITED STATES: CONSIDERATIONS FOR PHYLOGENETIC CHARACTERIZATION OF WILDLIFE AND DOMESTIC ANIMAL SPECIMENS

Emily Pieracci, Richard Chipman, Jesse Blanton, Clint Morgan, Jordona Kirby, Kathleen Nelson, Zachary Weiner, Andres Velasco-Villa, Elizabeth LeMasters, Xiaoyue Ma, Ryan Wallace

Presenter: Emily Pieracci

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Rabies virus (RABV) has been shown to spread among species and cross geographic boundaries, placing domestic animals and humans at risk, and justifying a need to develop a systematic approach to monitor RABV in animals in the United States. The goal of this evaluation was to identify high-interest samples that should undergo phylogenetic characterization to facilitate wildlife rabies management and aid in detection of host-shift events, translocation events, and introduction of novel variants. Secondarily we aimed to estimate the increased number of samples that public health laboratories could expect if all high-interest samples underwent phylogenetic characterization.

A modified Delphi method involving a panel of rabies experts was used to develop high-interest criteria for animal samples that should undergo phylogenetic characterization. Applying the criteria to animal surveillance data submitted to the Centers for Disease Control and Prevention during 2010-2015, and 2016 U.S. Department of Agriculture enhanced surveillance data, we analyzed testing trends and estimated the sample increase public health laboratories could expect annually.

Eleven high-interest criteria were identified by rabies subject matter experts. During 2010-2015, 3,827 animals met the high-interest characterization criteria; 1,601 (41.8%) had variant results reported. An estimated increase of 427 samples can be expected annually nationwide if public health laboratories characterize all terrestrial samples that meet at least one of the high-interest criteria. An estimated 335 (95%CI, 286.3-385.0) samples submitted annually were identified that had undergone variant characterization, but were not considered high-interest.

The high-interest criteria testing algorithm would help refine wildlife rabies management focus in the U.S. in real-time, allow for enhanced detection of novel RABV variants introduced into the U.S., and detection of host-shift and translocation events. Scarcity of variant data may be a reporting issue rather than a lack of testing. RABV variant trends should be monitored and reassessed frequently to ensure testing recommendations address knowledge gaps and ensure excessive testing does not strain public health laboratory capacity.

ANALYSIS OF A RABIES CROSS-SPECIES TRANSMISSION SUGGESTS A ROLE FOR SUB-VIRAL POPULATIONS IN SUCCESSFUL MAINTENANCE WITHIN NEW HOST RESERVOIRS

Denise A. Marston, Daniel L. Horton, Javier Nunez, Richard J. Ellis, Richard J. Orton, Nicholas Johnson, Ashley C. Banyard, Lorraine M. McElhinney, Conrad M. Freuling, Müge Firat, Nil Unal, Thomas Müller, Xavier de Lamballerie, Anthony R. Fooks

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Cross species transmission (CST) events play an important role in epizootics as adaptation to new hosts can profoundly affect the spread of the disease and the measures needed to control it. During the late 1990s, an epizootic in Turkey resulted in a sustained maintenance of RABV within the fox population. Utilisation of Bayesian inferences to investigate whole genome sequences from a cohort of fox and dog brain tissues from Turkey sampled demonstrated that the initial CST occurred in 1997 (+/- 1 year). Furthermore, these data indicate that the most likely source was from locally infected domestic dogs, rather than an incursion of a novel fox or dog RABV. No evidence was detected for virus adaptation to foxes, at consensus sequence level; therefore, the deep sequence data was analysed to investigate the influence of sub-consensus populations on virus adaptation. Viral heterogeneity was measured in all RABV samples; viruses in the epizootic stage had increased heterogeneity, in relation to those in the later enzootic stage, suggesting this could be a mechanism contributing to viral adaptation in new hosts. The dynamics of majority and minority variants are consistent with genetic drift, rather than positive selection. The expansion of sub-consensus viral populations in the new host species is hypothesised to enable the virus to overcome barriers, which otherwise would inhibit the successful onward maintenance in the new host.

COMMUNITY ENGAGEMENT AND DOG BITE PREVENTION

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Background - Cultural and social differences exist between remote Canadian indigenous communities and urban centres; dog populations are primarily unrestricted, semi-feral or free-roaming. Unrestrained dogs cause an average of 1-2 fatal dog attacks per year, and a considerable number of non-fatal injuries. An understanding of bite prevention can significantly decrease the incidence of dog bites, and reduce the potential for zoonotic disease transmission.

Methods – A mixed methods study, including a comprehensive scoping review on dog bite epidemiology, prevention and sequelae, was completed. Methods of community-driven policy creation and implementation were recorded, potential management plans and strategies were followed, and each alternative was evaluated for successful reduction in dog bites and violent dog-human encounters. These findings were then compared against the results obtained from the scoping review to determine similarities and differences in bite risks and successful interventions between northern Canadian communities and other recorded communities.

Results – Remote indigenous communities face significant challenges at all levels of dog health and population management. No single model is effective in every situation. Policy development, disease prevention and population restriction measures surrounding dog control and bite avoidance vary significantly between communities, and frequently depend on perceived threats of imminent aggressive encounters. Sustainability of interventions requires full community acceptance and support, in addition to access to required resources, especially appropriate education and training.

Conclusions - Communities create the most successful bite prevention interventions when working together to determine the main issues, and identify possible long-term solutions and education methods. In addition, involving community members in research and data collection provides the opportunity to appreciate the scope and breadth of potential problems and community opinions. Comprehensive community-initiated education has the potential to dramatically decrease the number of aggressive dog-human encounters that occur in indigenous communities in Canada.

DOG ECOLOGY AND ITS IMPLICATIONS FOR RABIES CONTROL IN GWAGWALADA, FEDERAL CAPITAL TERRITORY, ABUJA, NIGERIA

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The objectives of this study were to determine the characteristics of a dog population, including their accessibility to vaccination and healthcare, in urban and semi-urban areas of Gwagwalada, Abuja, Nigeria. Direct street counts and a house-to-house survey of city streets were performed. A total of 451 households were surveyed comprising 43.7% urban and 53.3% semi-urban areas. A total of 848 owned dogs were identified, along with 3,115 corresponding humans. With a dog-to-human ratio of 1:3.7, the dog population in the study area was estimated as 103,758. A total of 396 dogs were counted on the streets with the greater proportion (74%) in semi-urban areas. Most dogs in semi-urban areas (77.3%) had no certificate confirming vaccination against rabies, compared to 47.2% in urban areas ($p=0.004$). The majority of dogs in the urban (60.9%) and semi-urban (82.0%) were free roaming. In the multivariable model, age, presence of a collar, region, sex, use and having ever visited a veterinarian were significantly associated with rabies vaccination. The majority (125/197, 63.5%) of respondents with higher education were willing to pay more for the healthcare needs of their dogs as opposed to those with a lower level of education (93/251, 37.1%, $P= 0.001$). The study revealed a high dog population density, vaccination coverage below WHO recommendation of 70% and generally reduced healthcare seeking behavior amongst dog owners in Gwagwalada, Abuja, Nigeria.

EPIDEMIOLOGY OF HUMAN RABIES DEATH IN VIETNAM 1994-2016

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Human rabies deaths are preventable through prompt administration of post-exposure prophylaxis (PEP) after exposure to rabid animals. In Vietnam, imported and domestically produced equine rabies immunoglobulin are made available at all provincial medical centers and four regional institutes. Bite victims can go to any center for care, and are responsible for PEP associated costs. While PEP is widely available, approximately 91-300 dog-mediated rabies deaths still occur each year. To better understand the epidemiology behind these deaths, we examined investigation forms of probable cases (clinical definition and documented animal exposure) reported to the Ministry of Health between 1994-2016. There were 2,862 human rabies cases over the 22 year period. The average median age was 33 years old (range: <1-86). 99% of these deaths involved a dog exposure. 51% of families reported that the case-patients did not believe rabies vaccination was needed. 28% of families reported the case-patients seeking care from a traditional healer to treat rabies. Only 3% of families reported money was a reason for not seeking PEP.



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**POSTER PRESENTATIONS/PRÉSENTATIONS
D'AFFICHES/PRESENTACIONES DE
CARTELES/APRESENTAÇÕES DE PÔSTERES**

ANALYSIS OF THE VIRULENCE OF STREET RABIES VIRUS STRAINS ASSOCIATED WITH *CERDOCYON THOUS*

Fuoco NL, Silva NU, Ribeiro OG, Katz ISS

Presenter: Iana Katz

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The rabies cases in wild canids, *Cerdocyon thous*, in Northeastern Brazil are a public and animal health problem. Modifications in ecological conditions, such as deforestation lead to increased contact with wild and domestic animals and humans. In addition, *Cerdocyon thous* represents a major challenge for public health, as it travels widely, potentially helping to propagate RABV to regions where the disease is under control. The aim of this study was analyzed the virulence of RABV isolates from *Cerdocyon thous*. For this propose, N2a cells were infected with RABV isolated from *Cerdocyon thous* at a multiplicity of infection of 0.01. We observed that isolates from Ceará state replicated significantly better than isolates from Bahia state. These results indicate that two distinct sublineages could be circulating within the *Cerdocyon thous* host. Support for this hypothesis has been an 1166 km geographic distance between samples these states. In addition, recent study verified intragroup genetic distance among the isolates from canids (Souza et al., 2016, Arch Virol. 2017 Jan;162(1):71-77). This difference among the isolates could be related to the biological diversity of ecosystems in this region of Northeast in consequence of local selective pressures are exerted. Thus, we can deduce that there are distinct replication patterns for isolates that circulate among *Cerdocyon thous* in consequence of local selective pressures on the RABV genes caused by the reservoir's intracellular proteins involved in the viral replication cycle. These results can contribute to a better understanding of the RABV virulence of isolates from *Cerdocyon thous*, which is useful for plan strategies to control rabies.

IMPACT OF THE INITIAL STREET RABV AMOUNT IN THE BRAIN IN VIRUS REPLICATION

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Street rabies virus strains differ greatly in their lethality for adult mice, and the factors governing the regulation of rabies virus pathogenicity appear to be diverse and variable, depending on virus growth conditions and virus strain. Here we investigated impact of the initial street RABV amount in the brain in virus replication and internalization in cell culture. Virus titer in the original samples brain and in the supernatant after consecutive passages in NA cells was determined. The virus obtained from the seventh N2a cell passage was subjected to assays for

virus growth curves and kinetics of RABV internalization. We noted that high initial titer concentration (group II) in original sample was unfavorable for virus replication, when compared to low initial titer sample (group I). In addition, group I samples showed higher replication rate and enhanced internalization efficiency in N2a cells than group II. This could indicate that the dominant virus subpopulation selected in group II, high initial titer in brain, could promote adaptations advantage for the virus, allowing necessary for more efficient spread in the new host. Understanding the exact features of viral replication has a major impact in virus diagnosis, high virus yields in during vaccine production, as well as the pathogenesis of rabies.

ANALYSIS OF THE VIRULENCE OF STREET RABIES VIRUS STRAINS IN BHK CELLS

Nayara Ugeda Silva, Natalia Langenfeld Fuoco, Orlando Garcia Ribeiro, Iana Suly Santos Katz

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Rabies virus (RABV) has the ability to infect all mammals, but only a few species are important as reservoirs for the disease. RABV isolated from hematophagous bats (variant 3) exhibits longer incubation period, evolution period longer and a higher replication rate into neuroblastoma (N2a) cells, comparing to RABV isolated from marmoset (variant M) and dog (variant 2). Susceptibility of the baby-hamster kidney-cell line (BHK-21) to infection with RABV are also demonstrated. In this context RABV strains originating from bats and dogs could exhibit differences in glycoproteins, thus using different receptors to infect cells. However, there has been much speculation concerning the existence of particular RABV receptors. Therefore, this study was conducted to better understand the RABV replication in BHK-21 cells in focusing on the antigenic variants isolated from different reservoirs in Brazil. For this, after replication of the virus in BHK-21 cells, were performed growth kinetics of RABV samples isolates from marmoset, dog and hematophagous bats. We observed that the V-2 strain showed higher replication rate than V-3 and V-M. These results show that there is a replication rate variability on antigenic variants isolated from different reservoirs in Brazil. The replication results variability observed among BHK-21 cells and N2a cells may be explain by different receptors expression. These results can contribute to a better understanding of the virulence of the RABV maintained in different reservoirs in Brazil.

EXPRESSION OF CELLULAR RECEPTORS IN NERVE TISSUE OF MICE INFECTED WITH RABIES LYSSVIRUSES

Fernanda Monik Silva Martins, Jorge Rodrigues de Sousa, Alexandre do Rosário Casseb, Taciana Fernandes Souza Barbosa Coelho, Armando Souza Pereira, Francisco Hamilton dos Santos Paiva, Elizabeth Salb  Travassos da Rosa , Livia Medeiros Neves Casseb.

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The rabies virus presents some mechanisms of escape of the immune system, for example, the limitation of the local inflammatory mechanism by the expression of CD47, CD22 and CD200. This study allowed to verify the expression of the S-100, TLR-7 and MHC-II receptors against the experimental RABV infection, according to cellular expression in immunohistochemistry test. Samples of 225 mice previously inoculated with variants 2 (VAg2) and 3 (VAg3), and a control group, were analyzed through the via intracerebral, intramuscular and anterior intramuscular routes, and the animals were observed for 30 days. Afterwards, three slides of each block were made according to the immunohistochemical technique. This analysis was done by random counting of 10 regions in each encephalic compartment, comprising meningeal (M), parenchyma (PQ) and perivascular (PV). The ANOVA test was implemented with Bonferroni post-test to verify the difference between the receptor expressions. The s100 receptor presented expression in the meningeal, parenchymal and perivascular groups in the control group, perceiving its almost absence when the animal was inoculated by the intracerebral route with VAg2 and VAg3, also revealing a statistically significant difference ($p \leq 0.05$) between the compartments on all blades except when inoculated with VAg3 via intracerebral ($p = 0.46$). The TLR-7 receptor showed marking expression in the parenchyma only in the groups infected with VAg2 and VAg3, diverging from the control group, which did not show marking. The MHC-II receptor has no expression on any blade, and in no compartment, due to the fact that it is necessary to express the helper T lymphocytes, which do not exist in inflammations per rabies in the brain. Based on these results, it was possible to observe that RABV when is inoculated IM uses escape mechanisms, such as suppression of MHC-II and S100 expression, important for viral recognition.

ENGAGING RURAL STUDENTS IN BIOMEDICAL RESEARCH THROUGH ONE HEALTH FOCUSED UNDERGRADUATE RESEARCH EXPERIENCES

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Rabies is a quintessential One Health problem that requires integration of human and animal health approaches. The large size of Alaska and resulting subsistence lifestyle is a challenge for traditional western-based approaches to student engagement. By presenting biomedicine in the context of the One Health Paradigm, which explicitly links animal, environmental and human health, we hope to be able to specifically engage and retain underrepresented students into this program that synergistically integrates research and teaching and aligns more closely with indigenous patterns of learning and teaching. Our Biomedical Learning and Student Training program (BLaST) provides undergraduate research experience in a One Health context to train students in a meaningful way. Initial quantitative and qualitative data from students and faculty indicate high levels of engagement and satisfaction with mentored research experiences. Undergraduate researchers report significantly increased interest, comfort, and competency in laboratory research, and improved understanding of science and of laboratory research methods ($p < 0.01$ in all cases; Wilcoxon Paired Sample Tests). Further, these improvements were observed each semester of student experience – undergraduate research experiences continue to be perceived as learning experiences by students through successive semesters of participation. Trends suggest undergraduate researchers from rural backgrounds are especially interested in connections between animal/environmental health and human health. Together our research suggest that the One Health concept which essential in controlling rabies is a valuable tool to engage students in research and make this research more meaningful especially for students from a rural background that are underrepresented in biomedical research.



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**HUMAN RABIES AND PROPHYLAXIS/LA RAGE HUMAINE
ET LA PROPHYLAXIC/RABIA HUMANA Y
PROFILAXIS/RAIVA HUMANA E PROFILAXIA**

**POSTER PRESENTATIONS/PRÉSENTATIONS
D'AFFICHES/PRESENTACIONES DE
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COMPARISON OF IMPURITIES IN THREE HRIG PRODUCTS: PRELIMINARY RESULTS

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Human Anti-Rabies Immuno-globulin (HRIG) products (KedRAB, Kamada; HyperRAB[®], Grifols and Imogam[®], Sanofi-Pasteur) were compared for the presence of activated coagulation factor XI (FXIa). KedRAB and HyperRAB[®] were further compared for content of Immuno-globulin A (IgA) and IgG aggregates. KedRAB was found to have significantly lower contents of FXIa and IgA, and a lower percentage of IgG aggregates. These results may be attributed to KedRAB unique production process. Introduction: HRIG products contain impurities originating from the donors' plasma or the process. These may include activated coagulation factors, such as FXIa; IgA, which may elicit undesirable immune response in IgA deficient patients, and IgG aggregates, which may be involved in anti-drug-antibodies formation and Immunogenicity, and have been considered a reason for adverse reactions associated with IVIG.

Methods: The percentage of IgG aggregates was determined by SEC-HPLC. IgA content was established by a Quantitative nephelometry test (Minineph, The Binding Site). FXIa was tested using ROX FXIa Chromogenic Assay kit (Rossix AB, Mölndal, Sweden).

Results: KedRAB had a fundamentally lower content of FXIa (0.36 for KedRAB, vs. >100 mU/ml for both HyperRAB[®] and Imogam[®]); undetectable IgA levels (<0.03 mg/ml for KedRAB, vs. 0.23 for HyperRAB[®]) and IgG aggregate percentage of 0.6% for KedRAB vs. 1.6% for HyperRAB[®].

Discussion: KedRAB improved purity profile may be attributed to its unique production process which differs from the Cohn fractionation-based processes utilized by other manufacturers. Removal of impurities is paramount for the reduction of risks associated with the IgG preparation use, such as thromboembolic events, hypersensitivity reactions and immunogenicity.

EVALUATION OF THE PARTICIPATION OF THE VIRTUAL COURSE OF PROPHYLAXIS POST EXPOSURE OF RABIES OF PAN AMERICAN HEALTH ORGANIZATION/ WORLD HEALTH ORGANIZATION (DECEMBER 2015 - JUNE 2017)

Chávez O., Falcón N., Vigilato M.

Presenter: Ornela Chavez Inagaki

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The "Post-exposure Rabies Prophylaxis Self-Learning Virtual Course", implemented by Pan American Health Organization/ World Health Organization in December 2015, is made up of three modules and is provided free permanently, to guide and reinforce the technical knowledge and skills of the staff of the Americas countries on the management of prophylaxis against human rabies transmitted by dogs. From this, a retrospective study was carried out from the beginning of the course to June 2017. The objective was to perform a systematic quantitative analysis of the participants' information and their assessments.

4132 participants were enrolled, of which 2907 (70.4%) passed the course. Distribution by country was 17.6% (726) Mexico, 16.3% (674) Peru, 14.3% (589) Ecuador, 12.7% (524) Colombia, 2.2% (90) Argentina, 2.2% (59) Chile, 0.5% (22) Cuba, 0.5% (21) Brazil, 0.5% (20) Venezuela and the Dominican Republic, among other countries (1.7%). It was observed that countries with high rabies burden, such as Bolivia, recorded few participants (0.2%) as opposed to Peru (16.3%). 62.2% of participants were women; the median age was 31 years (IQR, 26-37); more than 90% were professionals and students in the health area. 99% of the participants considered relevant the topics and knowledge resources (readings, videos, among others) for their learning; 60.8% attended the course from home, 36.2% from work, 2.6% from public spaces, and 0.5% from their means of transportation. Some perceived disadvantages were related to hours of dedication (40.6%), use and complexity of the virtual platform (19.2%), poor internet access (8%), however, 37.1% had no problems accessing the course.

The assessment of the course demonstrates the success of the topics developed in the prophylaxis of rabies in the Americas for staff and students in the health area who have acquired up-to-date knowledge and skills.

EVALUATION OF POST-EXPOSURE PROPHYLAXIS IN VIETNAM

Houng Nguyen

Presenter: Huong Nguyen

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Vietnam has made monumental progress towards reducing canine-mediated human rabies deaths. Within a 22 year time frame, cases have dropped 82%, from 505 cases in 1994 to 91 cases in 2016. This reduction was largely due to the expanded access to rabies biologics throughout the country. While an average of 400,000 vaccine doses are administered and 32,000 people receive equine immunoglobulin (eRIG) each year, limited data exists regarding the procurement, distribution, storage, and administration of rabies biologics at the provincial and district level medical centers. Between February through June 2017, Vietnam's National Rabies Control Program surveyed 191 medical centers that order biologics directly from vaccine manufactures in 2016. Questionnaires were emailed or mailed to all 62 provincial medical centers (PMC) and 130 district medical centers (DMC). There was a 68.59% (62 PMCs and 69 DMCs, N=191 medical centers) response rate. 100% (131) medical facilities provided vaccine and 53.4% (70/131) provided eRIG. 43.5% (57) medical centers experienced delays in receiving vaccine or eRIG and 77 (58.8%) medical centers experienced a vaccine or eRIG shortage within the past year. Approximately 90% (118) medical centers used their internal funds to pay for biologics. Nineteen (15%) facilities depended on refrigerated space for other vaccines to store rabies biologics. Eight-seven medical facilities (66.4%) responded that lack of knowledge was the primary barrier for wound treatment after an animal exposure. Medical centers reported that financial cost (55%) to the patient and the distance from health facilities (31%) were reported barriers to getting rabies biologics.

EVALUACIÓN DE LA APLICACIÓN PARA SMARTPHONES SOBRE LA PROFILAXIS DE LA RABIA DE LA ORGANIZACIÓN PANAMERICANA DE LA SALUD/ORGANIZACIÓN MUNDIAL DE LA SALUD (ENERO-MAYO 2016)

Chávez O., Falcón N., Vigilato M.

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En los últimos años, se ha incrementado la generación de herramientas tecnológicas móviles que permiten ampliar las oportunidades de acceso a la información sanitaria para la mejora sostenible de la atención de salud. Por ello, la Organización Panamericana de la Salud/Organización Mundial de la Salud, desarrolló una «Aplicación para Smartphones sobre la Profilaxis de la Rabia» que contiene información basada en la Consulta de Expertos de la OMS sobre rabia, creada en enero de 2016 para guiar al personal de salud responsable de atender a personas expuestas al virus de la rabia en las Américas, con pautas para aplicar una adecuada

Profilaxis Post Exposición; encontrándose disponible en Play Store (Android) y App Store (iPhone), en español, inglés, francés y portugués. Se realizó un estudio retrospectivo, analizando sistemáticamente la información cuantitativa sobre las descargas de la Aplicación a nivel mundial durante 6 meses luego de su creación, observándose 37 descargas en Apple Store, los cuales fueron realizados en 27% (10) Perú, 18.9% (7) Brasil, 13.5% (5) México y Argentina. Sin embargo, la mayoría de descargas se realizaron con Play Store, registrándose 33,422 descargas de 27 países, con 17.9% (6009) Chile, 17.7% (5936) Colombia, 15.7% (5261) Perú, 10.1% (3400) Argentina, 8.9% (3005) México, 8.2% (2752) Brasil, 7.8% (2607) Bolivia, 1.7% (587) Ecuador, 1.2% (423) Estados Unidos, 1.2% (402) República Dominicana, 1.1% (395) Nicaragua, 1.4% (384) Uruguay, 1.08% (361) España. Entre otros países con menos de 1% de descargas se encuentran Tailandia, Panamá, Italia, Paraguay, Venezuela, Costa Rica, Trinidad y Tobago, Arabia Saudita, Canadá, Cuba, El Salvador, Guatemala y Sri Lanka (total 1875). La descarga masiva de la aplicación demuestra la importancia que tiene esta tecnología para disponer de material científico que puede usarse de referencia para la aplicación de medidas preventivas en países endémicos de rabia.

NEUTRALIZING ANTIRABIES ANTIBODIES PERSISTENCE AFTER SEVEN YEARS OF PRE-EXPOSITION RABIES VACCINATION

Regina Maria Mourão-Fuches, Adriana Brittes Pereira da Silva, Fábio Antônio Sena, Vlademir de Souza Pessoa and Neuza Maria Frazatti-Gallina - Rabies Laboratory - Butantan Institute

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Human rabies pre-exposure immunization is recommend for people in certain high-risk occupations such as laboratory workers handling live rabies and rabies-related (Lyssavirus) viruses. Workers of Butantan Institute involved in rabies vaccine and antirabies serum production and control or other activities carried out in these laboratories are vaccinate with three doses of Vero cells rabies vaccine on days 0, 7 and 28. A serological control, with titers of antirabies neutralizing antibodies is realize one or two times/year. In order to evaluate the immune response of these employers, four parameters were analyze time for a booster dose; relationship between the antibodies levels and protective titer (0.5 IU/ml), age and human gender influence in immune response. The neutralizing antibodies titers were determine by RFFIT. The results obtained in these tests showed that 19% of individuals needed a booster six months after primary vaccination, because there was an accentuated decreased of the titers (11.2 IU/ml to < 0.5 IU/ml for some individuals). After 12 months, there was a minor decrease of antibodies levels and only workers with titles below 2.0 IU/mL showed unsatisfactory titles. After 3 years 38% needed a booster and after 7 years 40%. The titers obtained in samples of male or female showed no significant difference. The analysis of the results found in employers with different age groups indicated there were no differences in the titers. In conclusion, the frequency for protective antibodies determination can be realize according the individual titers. It is very important for costs reduction because RFFIT is an expensive test.



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VACCINES AND ANTIVIRALS/VACCINS ET
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SAFETY AND IMMUNOGENICITY STUDY OF TLR-3 AGONIST-BASED RABIES VACCINE IN HEALTHY ADULTS

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Background: Human Rabies infection continues to be potentially fatal despite the availability of post exposure prophylaxis, due to the lack of immunoglobulin, poor vaccination compliance, insufficient immune response, and other reasons. A vaccine capable of producing protective immune response in a shorter time is needed. The PIKA Rabies vaccine, consisting of a TL3 agonist and inactivated purified rabies virus, demonstrated superior protection in compared with commercial vaccine in animal PEP models.

Methods: We conducted a phase I, open label, randomized study in healthy adults to assess the safety and immunogenicity of the PIKA Rabies vaccine and an accelerated vaccine regimen. Thirty-seven subjects were randomized into 3 groups: control vaccine classic regimen (n=12), PIKA vaccine classic regimen (n=13) and PIKA vaccine accelerated regimen (n=12). Subjects were followed up for safety, rabies virus neutralizing antibodies (RVNA) and T cell responses. Seroconversion is defined serum rabies virus neutralization antibody titer ≥ 0.5 IU/mL.

Results: No serious adverse events and deaths were reported. All AEs were mild in severity. Asymptomatic pyuria was the most frequent systemic reaction followed by presence of glucosuria, headache, diarrhoea, lethargy and proteinuria; the most common local AE is pain of site of injection. The AE profile is comparable between control and treatment groups.

All subject in the study seroconverted on Day 14. Seventy-five percent of subjects in the PIKA accelerated regimen seroconverted on day 7, compared to 53.9% in the PIKA classic regimen ($p = 0.411$) and 16.7% in control vaccine classic regimen ($p = 0.012$). The PIKA rabies vaccine elicited multi-specific rabies CD4 mediated T cell response already detectable ex vivo at day 7 after vaccination and that was maintained at day 42.

Conclusions: The investigational PIKA rabies vaccine was well tolerated and more immunogenic than the commercially available vaccine in healthy adults. Available vaccine in healthy adults.

EARLY POST-EXPOSURE ADMINISTRATION OF PIKA RABIES VACCINE DEMONSTRATES ENHANCED PROTECTION OVER COMMERCIAL VACCINE IN MOUSE MODELS

Hua Liu, Shi-rui Zhang, Yuan Liu, Yi Zhang, Zhongkai Shi

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Background: After Rabies exposure, the virus remains locally for at least 72 hours, while neutralizing antibody production by vaccination takes at least 3-7 days to occur. When rabies immunoglobulin is unavailable, early production of neutralizing antibody can help eradicate virus. PIKA, a synthetic stabilized form of double stranded RNA which interacts with TLR3, was employed as an adjuvant for the rabies vaccine.

Methods: Mice were challenged with a lethal dose of wild-type BD06 rabies virus, followed by immunization with PIKA rabies vaccine or controls. Both of the saline and commercial vaccine groups were vaccinated with classical 5-dose regimen (1-1-1-1-1) on days 0 (2 hr post-exposure), 3, 7, 14, 28. The third group was vaccinated with PIKA rabies vaccine using the 2-2-1 accelerated regimen on days 0, 2, 7 and divided into 12 subgroups, with first injection of each subgroup starting at different time points. Mice were monitored for survival. Brains from the succumbed mice were examined by rabies-specific immunofluorescence.

Result: Vaccination with PIKA rabies vaccine following the 2-2-1 regimen initiated from the 2nd, 12th, 24th hour post-challenge achieved survival rates of 43.3% (13/30), 46.7% (14/30) and 36.7% (11/30) respectively, significantly higher than that of the saline control group (3.3%). The protection rate did not exceed 6.7% when immunization began 48 hours or later post-challenge. Control vaccination with commercial rabies vaccine achieved survival rates comparable to mice vaccinated with saline (3.3% v 3.3%, $p > 0.05$). A dense coverage of rabies-specific fluorescence signals was detected in brains of all mice that died in the experiment. Brains from the mice survived through 30 days post-challenge and showed no rabies infection.

Conclusion: PIKA rabies vaccine, compared to commercial rabies vaccine greatly enhances viral clearance, and provides better protection in the early stage of post-exposure prophylaxis for rabies.



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DOMESTIC ANIMAL RABIES/RAGE DES ANIMAUX
DOMESTIQUES/RABIA ANIMAL DOMÉSTICA/RAIVA
ANIMAL DOMÉSTICA

POSTER PRESENTATIONS/PRÉSENTATIONS
D'AFFICHES/PRESENTACIONES DE
CARTELES/APRESENTAÇÕES DE PÔSTERES

WHAT IS THE IMPACT AND UPTAKE OF ONCE ANNUAL SUBSIDIZED VETERINARY SERVICES IN REMOTE COMMUNITIES IN THE NORTHWEST TERRITORIES?

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Dogs share a long history with Indigenous people in northern Canada, and remain important in daily life, although today, possibly more as companions than as working partners. Nonetheless, veterinary services remain inaccessible, unavailable and unaffordable for many Indigenous communities in northern Canada, which contributes to the human risk for zoonotic disease exposure, such as to rabies virus, which is endemic in Arctic Fox. In response to community concerns about dogs in the Sahtu Settlement Area of the Northwest Territories, the University of Calgary's Faculty of Veterinary Medicine has offered subsidized veterinary services to 5 Indigenous communities annually since 2008. The program offers core dog vaccinations, surgical sterilization, and dog husbandry and dog-bite prevention education. Providing such programs in an evidence based manner is important to a program's success, yet, subsidized veterinary programs are rarely evaluated. Our objective is to understand the uptake and impact of annual veterinary services in the communities over the last 10 years. Using chart reviews of the dog medical records from 2008-2017 and a dog census in each community we evaluated the rabies vaccination status of dogs over time. On program initiation, despite an existing government administered lay vaccinator program, only 37% of dogs seen at the clinics were vaccinated for rabies. This rate rapidly increased over the first few years of the program, and rabies vaccination rates in 2017 exceeded 70% of the dog population in most communities, and approached 90% in a few communities. Reflections on program efficacy and challenges acknowledge that differences between communities effect program uptake in each location. Recognizing these differences will improve the reach of the program in the communities and may help to establish similar culturally-sensitive programs in other poorly serviced areas of northern Canada.

EFFECTIVENESS OF HOUSE TO HOUSE STRATEGY OF DOG RABIES VACCINATION IN A PERI-URBAN SETTING IN THE PHILIPPINES

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Presenter: Daria Manalo

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The most effective strategy of eliminating rabies is still animal vaccination. The Philippines has been doing animal rabies vaccination since the early 1900s, however rabies remains a public health problem. A free house-to-house vaccination was performed by non-veterinarians in Muntinlupa City, a peri-urban area in Metro Manila in order to determine the effectiveness of this strategy as compared to the usual vaccination by post with registration fee. Health workers from small political units called barangays and other non-veterinary personnel were trained to conduct dog and cat vaccination and census. Vaccination teams composed of 3-4 personnel (1 or 2 vaccinators with 1 or 2 recorders) per team were assigned in different areas of the barangay to do house to house vaccination coupled with dog census. Every house was visited and homeowners were asked if they have pet dogs or cats and if they would like their animals to be vaccinated with anti-rabies, free of charge. All the vaccinated and unvaccinated dogs were recorded. From July to December 2016, eight (8) barangays were covered wherein most of the vaccination days were on Saturdays where the owners were present. The city census for dog and cat population was determined for the first time registering 26,860 dogs and 2,599 cats. The total number of dogs and cats vaccinated for 6 months was 26,517 reaching a vaccination coverage of 90.32%. The number of vaccinated animals was significantly higher than the previous vaccination of 16,728. In addition, there were 11 cases of animal rabies in 2016, as compared to only 4 cases in 2017. All these cases in 2017 were found out to be unvaccinated animals. It was shown from experience that a free of charge, house-to-house vaccination by trained non-veterinarians are very effective strategies in reaching the recommended coverage of more than 70%.

LEVEL OF ANTI-RABIES PROTECTION IN DOGS AND CATS WITH DIFFERENT NUMBER OF VACCINATIONS IN MARIQUITA COLOMBIA, AFTER AN OUTBREAK OF RABIES

María del Pilar Sánchez - Norma Patricia Gutiérrez

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In the last ten years, the Nacional Health Institute of Colombia has confirmed 17 cases of rabies in canines, 12 in felines and 16 in humans. The last human fatality was report in February this current year. They all were mortal victims of wild origin rabies, and the cat was the main transmitter.

Objective: To measure levels of protection with different number of vaccinations in dogs and cats from Mariquita, a region of Colombia where cases of canine and bovine rabies of wild origin were detected. **Methodology:** Sera samples of 130 dogs and 34 cats were analyzed with one, two, and three numbers of annual rabies vaccination, three years after a rabies outbreak occurred. The animals were randomly selected from the database of the Secretary of Health of Tolima using non-probabilistic sampling for convenience. The samples were processed utilizing the ELISA kit (Platelia Rabies), endorsed by the OIE for detection and in vitro titration of IgG antibodies against the virus glycoprotein. Only sera with titres greater than or equal to 0.5 IU/ml were OIE-protected. Results were analyzed using descriptive statistics using SPSS Statistical Software Version 23.

Results: Out of the 130 canine samples analyzed, 66 (51%) had one vaccination, 30 (23%) two and 34 (26%) three vaccinations for three years. The percentage of canines that had titres more or equal to 0.5 IU / ml, indicating an acceptable level of protection according to the OIE, were 38 %, 33%, and 50% with one, two, and three vaccinations respectively. In cats 20 (59%), 9 (26%) and 5 (15%) had one, two and three vaccinations with protection results for each one of 45%, 22% and 40% respectively. The low rates of protection especially in felines, and the high risk that this group represents, obligates a rigorous control and follow-up of vaccinations.

EVALUATION OF INTERFERENCE OF COMPLEMENT SYSTEM IN CELL CULTURE NEUTRALIZATION TESTS FOR TITRATION OF RABIES VIRUS NEUTRALIZING ANTIBODIES IN CAT SERA

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The main prophylactic form for animals susceptible to rabies is immunization since, in manifesting clinical signs, there is no effective treatment. Some European countries require an International Animal Health Certificate for the entry of the animal into the country of destination, so animals must present titers of rabies virus neutralizing antibodies (VNA) ≥ 0.50 IU/mL for adequate protection against rabies. A serological evaluation was carried out at the Pasteur Institute between 2006 and 2011, where a titre 10 times higher than the minimum required in the serum samples of felines was demonstrated. The inactivation of serum feline it is important because the complement system can continue to act after inactivation at 56°C for 30 minutes. The objective of this study was to evaluate the usual method of inactivation and complement system interference in samples of serum feline for the determination of neutralizing antibodies to rabies virus. Twenty-three serum samples of feline immunized against rabies were analyzed, aliquoted and inactivated at 30 minutes in a water bath at 56°C and 65°C and submitted to the Rapid Fluorescent Focus Inhibition Test to quantify rabies neutralizing antibodies. The results showed significant differences in titres obtained with inactivation for 30 minutes at 56°C (GM: 0.75 IU/mL, Max: 477.96 IU/mL, Min: 0.01 IU/mL), and the same samples when tested after inactivation at 65°C (GM: 0.25 IU/mL, Max: 47.8 IU/mL, Min: 0.03 IU/mL). At both temperatures nine serum had results <0.5 IU/mL. In conclusion the serum heat-inactivation at 65°C demonstrate efficient to inactivate complement system showing a decrease of VNA titers.

QUANTIFYING THE RISK OF RABIES IN BITING DOGS

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Rabies is a fatal viral disease typically transmitted through the bite of rabid animal. Domestic dogs cause over 99% of the human rabies deaths. Over half of the world's population lives in a country where the canine rabies virus variant is endemic and dog bites are common. An estimated 10 million people worldwide receive post-exposure prophylaxis (PEP) after being exposed to animals suspected of rabies. Accurate and timely risk assessment of rabies in biting dogs is critical to ensure that rabies PEP is administered more judiciously. In this study, a logistic regression model was developed to quantify the risk of rabies in biting dogs using data from Haiti's animal rabies surveillance program. Significant risk parameters identified were used to quantify the probability of rabies in biting dogs. The risk of rabies was highly increased when a biting dog displayed hypersalivation (OR = 34.6, 95% CI 11.3 – 106.5) or paralysis (OR = 19.0, 95% CI 4.8 – 74.8) and when the dog was dead at the time of the assessment (OR = 20.7, 95% CI 6.7 – 63.7). Lack of prior rabies vaccination, biting 2 or more people, and if the dog was a puppy also increased the probability that a biting dog would have rabies. The model showed high sensitivity (100%) and specificity (97%) when examined using validation data. This model enables us to precisely assess the risk of rabies in biting dogs in Haiti, and make preliminary PEP recommendations prior to laboratory testing and dog quarantine results. Application of this model may improve adherence to PEP for bite victims who can be educated on the quantitative risk of the exposure event. This model can also be used to reduce unnecessary PEP costs when the risk of rabies is determined as sufficiently low and the animal is available for observation.

RABIES VIRUS NEUROINVASIVENESS PATTERN IN THE CENTRAL NERVOUS SYSTEM FROM NATURALLY INFECTED CATTLE EVALUATED BY DIFFERENT DIAGNOSTIC TECHNIQUES

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Rabies can manifest itself in two ways: furious, characterized mainly by behavioral changes, and paralytic, most commonly observed in herbivorous animals, being more frequent the motor impairment. As there is a great variation of the symptoms, a laboratory confirmation of the clinical suspicion is necessary. However, the RABV does not affect all central nervous system (CNS) in the same way, thus choosing the best structure to be referred is imperative. Therefore, this study had as objective evaluate the viral distribution in the CNS different structures (cortex, cerebellum, hippocampus, thalamus, spinal cord and brainstem – bulb, pons and midbrain) by the use of the direct immunofluorescence technique (dFAT), immunohistochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR (RT-qPCR), based on the evaluation of 40 positive animals for rabies sent to Instituto Pasteur of Sao Paulo, totaling 210 analyzed fragments. The observed results indicate that there is no difference between bovine CNS structures regarding the intensity of antigenic labeling in the IHC technique. The same does not occur in the dFAT, since there was a statistically significant difference between the midbrain and the pons ($p = 0.0391$) and between the pons and the thalamus ($p = 0.0161$). The results obtained by molecular techniques indicate a lower sensitivity for detection of viral RNA in the cerebellum and thalamus. Considering the results obtained, regarding the techniques using antigenic labeling (dFAT and IHC), the fragments did not differ concerning the presence of the viral antigen. The confirmation of positivity by molecular techniques should preferably be obtained from the structures that compose the brainstem (bulb, pons, and midbrain). Institutions: School of Veterinary Medicine and Animal Science, University of Sao Paulo, Brazil. Instituto Pasteur de Sao Paulo, Brazil. Financial Support: Sao Paulo Research Foundation (FAPESP), process number 15/17807-0.



**BAT RABIES/RAGE DE CHAUVÉ-SOURIS/RABIA DE
MURCIÉLAGO/RAIVA DO MORCEGOS**

**POSTER PRESENTATIONS/PRÉSENTATIONS
D’AFFICHES/PRESENTACIONES DE
CARTELES/APRESENTAÇÕES DE PÔSTERES**

THE IMPORTANCE OF PASSIVE SURVEILLANCE: THE SECOND CASE OF RABIES BAT IN THE URBAN AREA OF JABOTICABAL, SÃO PAULO, BRAZIL

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Until the 1990s, the dog was the main responsible for maintaining urban rabies in Brazil. The campaigns of vaccination of dogs and cats made possible the control of the disease, being the dog variant considered practically eliminated. Since 2004, bats, hematophagous and non-hematophagous, have gained greater importance in the transmission of rabies to humans. Many municipalities started to perform the passive surveillance, leading the population to report the Service of Surveillance of Zoonoses (SVZ) when they find fallen bats. The objective of this study was to report the second case of rabies virus isolation from a chiropter in Jaboticabal, 14 months after the first positive case. It's about an *Artibeus lituratus* found dead on July 11, 2017 in the backyard of a residence, 3km from the place of the first case (*Artibeus planirostris* - AgV3). The animal was collected by the SVZ and sent to the Pasteur Institute of São Paulo, resulting as positive for rabies. For many years the SVZ of Jaboticabal has been forwarded bats to verify viral circulation. In 2015, the Office of Agricultural Defense (EDA) notified two cases of bovine rabies on the border of Barrinha Municipality at 20km; and in 2016, a case in Monte Alto Municipality at 25km. Jaboticabal is located 58km from Ribeirão Preto, where the rabies virus in bats and, eventually, in dogs and cats is frequently detected. There are no reports of human rabies in Jaboticabal and the last report of canine rabies was in 1982. Faced with the positive diagnosis in two bats in the urban zone in 14 months, and the recent cases of cattle rabies in neighboring municipalities, actions of surveillance for rabies prevention are being intensified by the SVZ and the EDA, including the municipal care, health education and increased vaccination coverage in dogs and cats.

THE IMPORTANCE OF PHYLOGENETIC ANALYSIS OF RABIES VIRUS ISOLATED FROM INSECTIVOROUS BATS *Nyctinomops laticaudatus* IN SÃO PAULO STATE, BRAZIL

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In Brazil, dogs and bats are the principal rabies transmitters in urban area. According to the Ministry of Health, bats account for about 12% of the cases of human rabies transmission in the country. Since 1998, in São Paulo State, virus isolated from dogs and cats has been identified as belonging to vampire bats *Desmodus rotundus* or other insectivorous or frugivorous species, which warns to the importance of bats in the transmission of rabies. Recent advances in epidemiological studies, based on the molecular analysis of the virus, have allowed to know its origin and its reservoirs. Given the importance of bats in the urban area, in particular the insectivorous, important natural reservoirs, especially in the Americas, living close to humans and their cats and dogs, the present study aimed to perform a phylogenetic analysis of the rabies virus from different municipalities of São Paulo State, identified as positive by Pasteur Institute, São Paulo. Partial sequences of N protein gene from 25 insectivorous bats *Nyctinomops laticaudatus* were analyzed. Samples were grouped in the Related Bat (VB) clade, but were divided into three subgroups according to the genetic relationship they demonstrated with each other. Virus from three samples were genetically related to the virus that are circulating in other species of frugivorous/hematophagous bats, including the genus *Artibeus* spp., common in urban areas in Brazil. The data indicate that there is coexistence between bats colonies in the same space, allowing the virus transmission among different bats species. Thus, given the diversity of virus lineages and the genetic relationship between them, the importance of studies with this nature is reinforced in order to contribute to measures of rabies control and prevention.

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RABIA EN MURCIÉLAGOS DE COAHUILA

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Debido a la amplia biodiversidad que caracteriza a nuestro país se ha detectado la presencia de diferentes ciclos de la rabia, presentándose además importantes desplazamientos de las especies de reservorios a otras áreas geográficas del país por diversas razones, pero especialmente por la intervención del ser humano. En el laboratorio de rabia del Laboratorio Estatal de Salud Pública (LESP) Coahuila, en coordinación con el Programa de Zoonosis de la Secretaria de Salud en la entidad, se ha realizado el monitoreo de virus rábico en distintas especies de animales silvestres, entre ellos coyote, zorro, mapache, tejón y diversos géneros de quiróptero, siendo estos últimos los de mayor interés ya que es en ellos donde se han presenten casos positivos en algunas especies de insectívoros. De acuerdo a la Asociación Mexicana de Mastozoología, en el estado habitan más de 25 especies diferentes de murciélago insectívoro, de las cuales en el laboratorio tenemos identificadas 13, entre las cuales hemos tenido 5 casos positivos, 2 de ellos en *Tadarida brasiliensis* de los municipios de Cuatro cienegas y Progreso, 1 en *Lasiurus cinereus* del municipio de Saltillo, y 2 más de los municipios de Piedras Negras que no pudieron ser identificados debido a que no se recibió el espécimen y solo enviaron el encéfalo. Todas estas muestras fueron enviadas al InDRE para confirmación por IFD, caracterización antigénica y genética del virus y se está en espera de recibir los resultados de la confirmación de casos y de pruebas de identificación de las variantes antigénicas de virus rábico identificadas en estas especies, para integrarlas a los bancos genéticos correspondientes.

SPECIES IDENTIFICATION OF RABIES POSITIVE BATS IN SÃO PAULO STATE – BRAZIL, FROM JANUARY/2015 TO JUNE/2017

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Bats found in Brazil are usually small animals with different eating habits and are distributed in nine families, 68 genera and 178 species. In this country, at least 42 species with positive diagnosis for rabies were registered; belonging to 25 genera and three families. This study aimed to identify bat species with positive results for rabies during the period from January/2015 to June/2017. During this period 6,033 bat specimens were received, which were submitted to diagnostic techniques for rabies virus recommended by WHO. Of these, 114 (1.89%) specimens resulted positive for rabies in one or more techniques performed in the Virology Section of Instituto Pasteur – São Paulo - Brazil. These animals were identified in family, genus and species by morphological and morphometric analysis using an identification key. In addition, they were classified as feeding habits. The identified bats belonged to 3 families, 12 genera and 17 species. The family with the highest number of positive specimens was Phyllostomidae (45 animals), followed by Molossidae (31) and Vespertilionidae (28). The positive species were *Artibeus lituratus* (36), *Artibeus planirostris* (4), *Desmodus rotundus* (4), *Sturnira lilium* (1) from the Phyllostomidae family; *Nyctinomops laticaudatus* (9), *Molossus molossus* (3), *Eumops glaucinus* (3), *Cynomops planirostris* (2), *Tadarida brasiliensis* (1), from Molossidae family and *Eptesicus furinalis* (13), *Myotis nigricans* (11), *Myotis albescens* (10), *Histiotus velatus* (4), *Lasiurus cinereus* (2), *Lasiurus ega* (1) from Vespertilionidae family. Ten specimens were unable to identify. Regarding the feeding habit, 59 specimens were insectivorous, 41 were frugivorous and 4 were hematophagous. The results show the importance of these bat species in rabies virus circulation in São Paulo State. The knowledge of the species reservoirs, including their feeding habits, is important to epidemiological surveillance measures for rabies control.



DIAGNOSTICS/DIAGNÓSTICOS

POSTER PRESENTATIONS/PRÉSENTATIONS
D'AFFICHES/PRESENTACIONES DE
CARTELES/APRESENTAÇÕES DE PÔSTERES

INNOVACIÓN EN BIOSEGURIDAD EN LA SUJECCIÓN DE CABEZAS PARA LA EXTRACCIÓN DE ENCÉFALOS EN EL DIAGNÓSTICO DE RABIA

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La calidad de una muestra determina la calidad de un resultado de laboratorio y el diagnóstico de rabia no es la excepción. La extracción de encéfalo de cabezas de animales sospechosos de rabia es un paso primordial para realizar el diagnóstico de esta enfermedad. Las medidas de bioseguridad y la facilidad de extracción determinan la seguridad del personal y la calidad de la muestra, por lo que es necesario utilizar equipo de protección durante el proceso y solucionar posibles inconvenientes como el que la cabeza del espécimen tenga movilidad durante el corte de cráneo. En algunos casos, dicha movilidad ha dificultado la toma de la muestra y provocado accidentes. Cuando se reciben cabezas de animales domésticos en los que existió exposición hacia personas, el procedimiento debe realizarse a la brevedad para obtener un resultado oportuno. En el Centro Nacional de Servicios de Diagnóstico de Salud Animal (CENASA) se realizan las técnicas de inmunofluorescencia (FAT), titulación de anticuerpos antirrábicos (FAVN), aislamiento viral en cultivo celular (RTCIT), inoculación en ratones y constatación de vacunas, sin embargo, la preocupación por la seguridad del personal y el análisis de riesgo al tomar la muestra, nos ha llevado a diseñar una prensa que permite sujetar cabezas de gato, perro, borrego, cabra, vaca, caballo y algunas especies silvestres, la cual facilita la práctica común de extracción de encéfalos, reduce el riesgo de accidentes y permite obtener muestras íntegras. Su diseño hace que el corte del cráneo se realice fácilmente y con menor esfuerzo. Además, el instrumento se pueda lavar, desinfectar y esterilizar expeditamente. En conclusión, este equipo aporta beneficios durante la extracción de encéfalos: aumento en la bioseguridad al disminuir el riesgo de heridas, ahorro de tiempo, facilidad de trabajo y obtención de muestras de mejor calidad.

THE DEVELOPMENT OF THE HIGH THROUGHPUT NEUTRALIZATION ASSAY FOR ANTI-RABIES ANTIBODY DETECTION

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Virus neutralization assay is a part of essential assessment for vaccine efficacy. The rapid fluorescent focus inhibition test (RFFIT) currently used to measure rabies virus (RABV) neutralization antibodies is time consuming, labor intensive, and requires skilled personnel and containment facilities to perform. The development of a high-throughput neutralization test (HTNT) against RABV using a recombinant virus that expresses the green fluorescent protein (GFP) requires minimal amounts of material and provides a platform for rapid unbiased quantitation of RABV infection based on GFP fluorescence followed by virus entry and viral gene expression. Advantages include a high-throughput capability using either 96- or 384- well plates, allowing for more samples to be screened in one test. A panel of both human and animal serum samples were tested in both the HTNT as well as the traditional RFFIT assays. The focus of this study was to demonstrate effectiveness of high-throughput assay using large sero-surveillance samples to determine anti-rabies neutralizing antibodies. A comparative analysis of results obtained by RFFIT and HTNT will be presented.

DEVELOPMENT OF NEW HIGH AFFINITY MONOCLONAL ANTIBODY AGAINST RABIES VIRUS MATRIX PROTEIN FOR DIAGNOSIS OF HUMAN RABIES

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Rabies is one of fatal zoonotic disease in both human and animals. However, rapid and suitable pre and post exposure prophylaxis using rabies vaccine with or without human rabies immunoglobulin is highly effective in preventing disease. Therefore, quick and accurate diagnosis is also important for decreasing human death as well as economic burden. In the present study, we developed a new high affinity monoclonal antibody (mAb) against rabies virus matrix (M) protein from clinical isolate in Korea (KGH). Recombinant rabies M protein as an antigen was expressed by Escherichia coli system using pET-28b vector. The generated mAb 2D3

from mouse was applicable to the western blot analysis as well as the rapid fluorescent focus inhibition test which were gold standard method for measuring neutralizing antibody for rabies, and evaluated its utility as a diagnostic reagent. In addition, Alexa flour 488-conjugated mAb 2D3 showed highly performance about the detection of rabies antigen comparing to commercial available rabies DFA reagent in indirect immunofluorescence assay using CVS-11, ERA, or KGH infected BHK-21 cell slide. These results indicated that our new mAb would be available wide range of application from basic research areas to diagnosis of rabies antigen and antibodies. It is expected to contribute to increasing public health and economic benefit through improving diagnostic reagent. This study was supported by a research grant (2016-NG52001-00) of Korea Centers for Disease Control and Prevention.

UPDATE ON NEW RABIES DIAGNOSIS TECHNIQUES

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The rabies diagnosis in animals or humans generally consists in the analysis of a brain biopsy or brain specimen using conventional methods such as the Fluorescent Antibody Test (FAT) and the Rapid Tissue Culture Infection Test (RTCIT). Both techniques are the reference methods currently recommended by OIE and WHO. During the past years, many scientific publications, reports, guidelines promoted new techniques for a more rapid, more sensitive, cheaper and more affordable rabies diagnosis. Lateral Flow Assays (LFA) attracted considerable interest because of their ability to provide instantaneous diagnosis and a new “field use” approach to improve rabies surveillance systems in developing countries. The Direct Rapid Immunohistochemical Test (DRIT) is a novel assay for the demonstration of lyssavirus antigens, which appears to be as sensitive and specific than the gold standard FAT. The DRIT is also a promising tool for diagnosis laboratories that cannot perform the FAT easily. Molecular biology techniques have also benefited from substantial progress during the past decade and are accepted as a standard technique for many important viral pathogens. Molecular assays –conventional and real-time RT-PCR- are specific, rapid and more sensitive than the conventional tests. With pan-lyssavirus primers, all known species of lyssavirus can be detected. Using real-time RT-PCR, a large number of samples can be analyzed in less than 4 hours, giving an interesting and useful technique for the demonstration of rabies RNA. The potential of these new techniques, currently discussed at the OIE Biological standards Commission, as well as their future contribution in rabies diagnosis will be presented here.

NEUTRALIZING ACTIVITY OF PLANT-DERIVED RABIES MONOCLONAL ANTIBODY AGAINST CLINICAL RABIES ISOLATE FROM SOUTH KOREA

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Rabies is a zoonotic disease that transmits to human through the biting by rabies virus infected animals. Currently, more than 55,000 people died every year by rabies in the world and WHO recommend post-exposure prophylaxis (PEP) to prevent human rabies. In South Korea, no case has been occurred until now since 6 patients were died during 1999~2004 by biting of rabid raccoon or dog. However, animal bite patients were reported more than 2,300 cases for 3 years (2014~2016) in South Korea. It is necessary to develop new diagnostic or therapeutic agents of rabies virus because increasing animal biting caused threat of public health and economic burden of human immunoglobulin and preventive rabies vaccine for PEP. Previous study reported about the neutralizing activity of plant-derived monoclonal antibody (mAbP SO57) for CVS-11, standard rabies virus. Here, we confirmed neutralizing effect of mAbp SO57 against clinical rabies isolates from South Korea (KGH) by the rapid fluorescent focus inhibition test. In addition, the binding sites of mAbP SO57 on glycoprotein of KGH strain was analyzed by conformational epitope mapping. Four candidate sites were detected on KGH glycoprotein, and the major epitope was located in 195-202aa (SRGKRASK). mAbP SO57 could not detect KGH stain by indirect immunofluorescence assay using KGH infected N2a cell slides although it was known to detect CVS-11 strain. Taken together, these results suggested that mAbp SO57 would be applicable to the development of diagnostic agents, potential therapeutic agents, or effective vaccines for rabies in future. This study was supported by a research grant (2016-NG52001-00) of Korea Centers for Disease Control and Prevention.

PERFORMANCE EVALUATION OF LABORATORIES IN RABIES DIAGNOSIS TEST: WHICH TECHNIQUES LEAD TO THE MOST RELIABLE RESULTS IN PRACTICE?

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An inter-laboratory trial dedicated to rabies diagnosis techniques is organized annually by the European Union Reference Laboratory (EURL) for Rabies. The objective is to assess the technical performance of laboratories based on the rabies diagnosis reference techniques namely the Fluorescent Antibody Test (FAT) and the Rabies Tissue Culture Infection test (RTCIT) but also to compare results obtained with the current biological molecular techniques represented by the conventional and the Real Time RT-PCR. For the 2016 session, the lowest proportion of laboratories producing discordant results was found in the Real Time PCR test (3.7%), closely followed by RT-PCR (8.3%). The gold standard technique, the FAT, harboured 14% of laboratory with discordant results. For the second consecutive year, the Real time test was the trial with the best laboratory performance level. In contrast to previous years, false negative results were more frequent than false positive results in both FAT and Biological molecular techniques (RT-PCR and Real Time PCR). The GS7 diluted samples present higher percentage of discordant results in FAT while BBLV samples present higher percentage of discordant results in Real Time PCR. Last years of performance evaluations confirmed that, over time, very few false negative results were observed in RT-PCR compared to other techniques and that the RTCIT presents the less stable results and the lowest laboratory performances.

VARIABLE RABIES VIRUS DISTRIBUTION ACROSS DIFFERENT BRAIN REGIONS IN THREE 'LOW POSITIVE' CLINICAL SAMPLES

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During the first half of 2017, the Centers for Disease Control and Prevention tested over 100 rabies suspect cases sent for confirmatory testing from state public health labs (SPHLs) and other sources. One sample of particular interest was identified as negative by the gold standard direct fluorescence antibody (DFA) test but positive by real-time PCR at the SPHL. This sample was positive by both DFA and real-time PCR when re-tested at the Centers for Disease Control with relatively low, remarkably variable antigen distribution. Two additional rabies confirmatory samples received in 2017 produced positive results by DFA in the brain stem but limited to no antigen in the cerebellum. Here, we present Rabies virus antigen distribution and RNA levels in samples from different brain regions of these three cases, as determined by the DFA test, the direct rapid immunohistochemical test (dRIT), and real-time reverse-transcription PCR. For all three tests, the highest level of antigen or RNA was detected in the brainstem. Estimated levels of Rabies virus RNA ranged from 3 to 500 fold more abundant in the brainstem compared to the cerebellum after normalizing for tissue amount using the housekeeping gene beta actin. Together, these findings highlight the importance of examining rabies virus antigen or RNA from ample tissue representing a full cross section of brain stem as well as the importance of molecular techniques for rabies diagnosis in samples with low virus load.

COMPARISON OF THE HIGH THROUGHPUT NEUTRALIZATION ASSAYS FOR RABIES ANTIBODY DETECTION USING DIFFERENT PLATFORMS

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Neutralization assays are the functional tests to demonstrate presence of anti-rabies virus specific antibodies. The traditional assay to determine neutralizing antibodies against rabies virus (RABV) is the cell-based rapid fluorescent focus inhibition test (RFFIT). The RFFIT, a labor intensive and time consuming test, necessitates skilled personnel and equipment. Some of the drawbacks of the RFFIT are low throughput, bias in reading and interpretation, and absence of recording results for future analysis. In order to address some of RFFIT's limitations, we developed high-throughput neutralization tests across two platforms. The high throughput assays utilize a recombinant RABV expressing green fluorescent protein (GFP) to assess the presence of rabies neutralizing antibodies in serum samples. Quantification and analysis of RABV infection based on GFP fluorescence were performed using either (1) a high-content screening (HCS) method that employs automated microscopy and multi-parameter image analysis or (2) flow cytometry to quantitate GFP expressing cells. The results from high-throughput assays using HCS, and flow cytometry were compared with traditional RFFIT assays. This study demonstrates the potential usefulness of developing and validating different high-throughput neutralization assays which allow laboratories the flexibility and ability to test large sample sets.

BASELINE FEATURES OF A RABIES VIRUS STRAIN ISOLATED FROM A *DESMODUS ROTUNDUS* BRAIN PERU 2014

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Seeking preliminary basic information to find a laboratory strain for a reference diagnosis and a specific human rabies vaccine production to control rabies due to vampire bat bite, a wildlife rabies virus field strain from a *Desmodus rotundus* brain sample was identified and isolated. Wildlife rabies is endemic in Peruvian jungle, *Desmodus rotundus* bat is the main host of rabies virus. During the last seven years in Peruvian jungle occurred 59 human rabies cases and 1035 animal cases due to vampire bat bite. Blood serum samples and one cerebrospinal fluid sample of native villagers bitten by bats during outbreaks in different communities of Peruvian jungle demonstrated the presence of rabies virus neutralizing antibodies. It is postulate that wild rabies virus strain is able to induce a natural resistance against rabies. A *Desmodus rotundus* was easily captured in the community of Roqchas district of San Miguel province of La Mar in Ayacucho, into an area in which days before a positive case of bovine rabies was diagnosed. Positive FAT RT – PCR and MIT confirmed rabies virus. Antigenic variant was determined with MAB test. Fluorescence microscopy image showed uniform distribution of antigen as rabies dust in 100% of fluorescent fields. RT- PCR obtains a 383 bp fragment, incubation period in the suckling mouse brain was 12 days, the infectivity titre was $10^{-2.6}$ DL50. antigenic variant was 5. The strain has own features of wild virus, by successive passages in mouse is able to become fixed virus, in order to increase the reliability of laboratory tests and the immune response after the vaccination to prevent rabies due to *Desmodus rotundus* bite.

RABIES VIRUS DETECTION AND TYPING USING PYROSEQUENCING

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Rabies, resulting from infection by Rabies virus (RABV) and related lyssaviruses, is one of the most deadly zoonotic diseases and is responsible for up to 70,000 estimated human deaths worldwide each year. Rapid and accurate laboratory diagnosis of rabies is essential for timely administration of post-exposure prophylaxis in humans and control of the disease in animals. Currently, only the direct fluorescent antibody (DFA) test is recommended for routine rabies diagnosis. In this study, we developed a rapid RABV and other lyssaviruses diagnostic and typing method by combining SYBR green RT-PCR and pyrosequencing of the RT-PCR amplicons. The target region of pyrosequencing is a 33 bp highly variable sequence located in the leader region of the lyssavirus genome. Compared to a traditional Sanger sequencing approach, this rapid protocol can generate PCR amplicons and sequences in 4 hours and allows a confirmation of diagnostic results based on sequences. The assay conditions were optimized to achieve longer reads and reliable base calling although degenerate PCR primers were used in the assay. The assay was validated using a panel of highly variable RABV and other lyssavirus samples which showed that the SYBR RT-PCR has improved sensitivity compared to Taqman real-time RT-PCR. Furthermore, the sequences provide sufficient resolution to differentiate most canine rabies lineages and other lyssaviruses. The assay was validated of its specificity and sensitivity using both postmortem and antemortem clinical samples in the CDC Rabies diagnostic lab.

EVALUATION OF THE ANALYTICAL SENSITIVITY OF THE VIRAL ISOLATION TECHNIQUE IN N2A CELL CULTURE USING DIFFERENT PROTOCOLS: PRELIMINARY RESULTS

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Virus isolation in cell culture (VICC) presents advantages when compared to viral isolation in mice, among which are the reduction of time to obtain the results, controlled environment and lower cost. Data from previous studies indicate that the use of suspensions in concentrations below to 20%(p/v) shows a better performance to detect the antigens of the rabies virus, as well as better preservation of the integrity of the confluence of the cellular monolayer. The aim of the present study was to evaluate whether there is a difference in the analytical sensitivity of the viral isolation technique in cell culture when two parameters were changed: volume and amount of cells. Three bovine and three equine samples, diagnosed positive for rabies by direct immunofluorescence test, and two CVS fixed virus samples from different batches were evaluated. Suspensions of 20%(p/v) concentration were prepared and all samples were submitted to viral titration in N2A cell culture, using the Reed & Muench method. To evaluate the analytical sensitivity, a threshold detection test was performed, so that the samples were serially diluted with a dilution factor of 10, inoculated in triplicate in 96-well plates, according to the protocols that had different sample volumes (5µl, 10µl, 20µl and 40µl) and amount of cells (5x10⁵cells/mL and 2.5x10⁵cells/mL). One equine sample showed no fluorescence, indicating there was no VICC. Titers ranged from <25X10 to 25X10⁸TCID₅₀/mL. Six samples (85.7%), when inoculated with a 40 µl volume, showed detection at a further dilution in relation to the volume of 5µl, with 5x10⁵ cells/mL. Considering the amount of cells inoculated, six samples (85.7%) inoculated with 2.5x10⁵ cells/mL showed an increase in the threshold detection in at least one of the volume protocol. The data suggest that reducing the amount of cells, we may increase the analytical sensitivity of the VICC technique.



**WILDLIFE RABIES/RAGE DE LA FAUNE/RABIA DE LA VIDA
SILVESTRE/RAIVA DOS ANIMAIS SELVAGENS**

**POSTER PRESENTATIONS/PRÉSENTATIONS
D'AFFICHES/PRESENTACIONES DE
CARTELES/APRESENTAÇÕES DE PÔSTERES**

GENETIC CHARACTERIZATION OF SPECIES OF WILD CANIDS IN THE STATE OF CEARA AND ITS CORRELATION WITH THE RABIES VIRUS

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Rabies in wildlife has emergent importance in Brazil and the northeast region presents a unique epidemiology compared to the rest of the country. In this region were identified the two variants of rabies virus that are maintained and transmitted by wild terrestrial animals. These viral variants were initially identified in Ceara State, one in marmosets (*Callithrix jacchus*) and the other in wild canids, being restricted to the Northeast region. The aim of this study was to identify genetically, using mitochondrial DNA, the wild canids species of occurrence in the Ceara State, that act as reservoirs and transmitters of the virus and the genetic characterization of isolated of the rabies virus from these animals. All samples were obtained from animals found dead or ill by passive surveillance. The study was carried using PCR, RT-PCR and genetic sequencing techniques. A total of 20 samples from wild canids were studied and 04 presented positivity to rabies virus. In the phylogenetic tree, all samples of wild canids segregated with the samples from *Cerdocyon thous*, considered the main species of occurrence in the region. Among the 04 isolates of rabies virus, all samples segregated in the genetic group formed by domestic and wild canids in a subgroup formed by wild canids from the northeast of Brazil. These results suggest that the crab eating fox (*Cerdocyon thous*) is the main wild canid of occurrence in Ceara State, being responsible for the maintenance and transmission of the rabies virus variant previously described as related to these animals in northeast of Brazil. These results can contribute to a better knowledge of the species involved in this epidemiological cycle of the disease, allowing the optimization of the actions for prevention and control of rabies in wildlife, considering the species characteristics and its interaction with humans and domestic animals.

EVIDENCE FOR LONG-TERM SURVIVAL OF ARCTIC FOXES FOLLOWING RABIES EXPOSURE

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Although rabies is endemic in the North American Arctic, our understanding of rabies dynamics in arctic foxes is predominantly generated from regions with a high probability of wildlife/human interactions. We report rabies exposure data in arctic foxes from a remote site in the central Canadian Arctic (Kitikmeot Region, Nunavut) that is ~300km away from human communities. We surveyed sera (n=59) from 41 individual arctic foxes (2011-2015) for rabies antibodies by competitive enzyme-linked immunosorbent assay (cELISA) and rabies virus neutralizing antibody test (RVNA). Through a long-term capture mark-recapture, we were able to complete multi-annual surveillance for 9 individual foxes. We detected rabies antibodies in 7/59 serum samples from unique individuals. The overall antibody prevalence of rabies virus arctic foxes at Karrak Lake captured at any time from 2011 to 2015 was 17% (seven of 41 individuals), combining results from either the cELISA or RVNA. We detected antibodies by cELISA in 6/59 samples and in 3/59 by RVNA. Sera from two individual foxes demonstrated detectable antibodies by both assays. One of the two foxes with test agreement was not recaptured after rabies antibodies were detected. The second fox with test agreement in 2014 was recaptured in 2015. Antibodies were not detected in the latter year, and the fox raised an apparently healthy litter that summer. While it is unknown how these foxes were exposed to rabies virus (i.e., through prey ingestion or bite wound), our results add to the available knowledge of arctic fox survival following general exposure to rabies virus, and provide preliminary evidence for the circulation of rabies virus in the Karrak Lake ecosystem.

PATTERNS OF HOST GENETIC STRUCTURE IN RELATION TO VIRUS VARIANT IN A RECENT FOX RABIES EPIZOOTIC OF THE EASTERN SUBARCTIC

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As part of an ArcticNet-funded project regarding wildlife diseases and public health in the Eastern Subarctic, we are analyzing the origin and spread of a recent fox rabies epizootic in Labrador. We report the population genetic structure of coloured and Arctic foxes across northern Canada, assessed with mtDNA control region sequences and microsatellites, and compare it with virus variant distribution, to better understand the movements of vector and virus across the landscape. Analysis of mtDNA of coloured foxes revealed some genetic structure on a broad scale but little genetic variation within Labrador and no host genetic structure among Arctic foxes. Microsatellite analysis provided finer scale resolution of host genetic structure in the Eastern Subarctic and was able to differentiate southeastern populations from each other and from western and northern locations. Viral genome sequencing identified a variety of sub-types of the arctic A3 lineage that circulated across Canada in the late 1990s and early 2000s. Where the fox host ranges overlapped both coloured and arctic foxes shared many of the same viral sub-types although some viruses did appear to be associated with one or the other host species. However from 2010 onwards only two subtypes circulated across northeastern Canada and only one of these was responsible for all cases from Labrador over this period. This work provides baseline data on fox population structures and correlation with virus variants that will inform modeling studies for the prediction of future fox rabies outbreaks. More broadly, we aim to provide information on zoonotic diseases in the North to help develop strategies for managing diseases affecting both wildlife and public health in the Arctic.

OPTIMIZING ORAL RABIES VACCINATION AERIAL DISTRIBUTIONS FOR STRIPED SKUNK

Shylo Johnson, Chad Blass, Sam Mills, Nikki Walker, Travis Flanagan, Justin Fisher, Kim Pepin and Kurt VerCauteren

Presenter: Shylo Johnson

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In the continental United States, several different terrestrial mammals are host to different rabies virus variants. Striped skunks (*Mephitis mephitis*) are one of these host species and are a common spillover host for other rabies variants. Achieving elimination of terrestrial rabies will require efficient oral rabies vaccination (ORV) targeting skunks. A targeted baiting strategy would maximize bait exposure for the target species while minimizing cost. For aerial ORV distribution, factors that can be modified are the distance between aircraft flight lines, spacing between baits along the flight line, and the orientation of the flight line. In 2014 and 2015, movement data were collected from 22 skunks concurrent with local ORV delivery. The mean core and overall home ranges for these skunks were 5.6 ha (± 3.7 ha SD) and 35.2 ha (± 24.0 ha SD), respectively. Using this movement data, we compared several different baiting scenarios to identify an optimal baiting strategy. We created scenarios based on flight line spacing ranging from 125 m to 750 m. We examined bait spacing along the flight line ranging from 9 m to 107 m to represent bait densities of 75, 150, and 300 baits/km² and examined flight orientation with lines parallel and perpendicular to the actual application. Each scenario covered 1118 km² and cost estimation for each scenario was based on the price per bait and cost per linear kilometer for aircraft travel along flight lines. Using unique home ranges for each skunk, we calculated the number of baits in core and overall home ranges for each scenario as a proxy for bait exposure. Trade-offs between cost and bait exposure will be discussed.

HUMAN EXPOSURE TO A NOVEL RABIES VIRUS VARIANT OF BAT ORIGIN THROUGH A FOX BITE REVEALS COMPLEX RABIES TRANSMISSION PATHWAYS

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Presenter: Andres Velasco-Villa

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A novel rabies virus variant of bat origin was detected in a rabid gray fox that attacked a 78 year old woman in the New Mexico. Standard rabies PEP was successful in this patient. This investigation aim to; provide robust genetic evidence to support the uniqueness of a putative novel rabies virus variant found in a gray fox in NM, infer its likely primary reservoir host, reveal potential spillovers or host shift events conducive to its emergence in a fox, and estimate a likely timeline of divergence from its closest relatives. Complete N gene sequences obtained from forty-seven samples recovered from NM and surrounding states were compared with 353 complete N gen sequences from GenBank and a CDC database, representing the current diversity of rabies viruses circulating the Americas. Likely origins and reservoir host for this novel variant were inferred with MEGA V7 and Garli 2.0 programs. Divergence time of the novel NM variant with its most close relatives was estimated with dated maximum clade credibility reconstructions using the random local clock model and duplicate Markov Chain Monte Carlo runs implemented in the program BEAST V 1.8.3. . The novel rabies virus variant identified in NM shares a common ancestor with several RABV variants associated with tree bats. A bat is the likely reservoir host of origin of this novel variant, from which it diverged nearly 400 years ago together with the common ancestor that gave raise to most of rabies virus variants independently associated with tree bats. Since then, circulation of this rabies virus has remained unrecognized as a host shift in gray foxes or stayed persistent in a bat reservoir host not yet identified. This investigation highlights complex evolutionary patterns of rabies virus and the surged of alternative transmission pathways that may eventually reach humans. Implementation of laboratory-based rabies surveillance systems for the sensitive detection of rabies viruses in their primary rabies reservoir hosts in a comprehensive geographic scale, may ultimately contribute to the improvement of rabies control strategies.

CORRECT USE OF THE CURRENT LYSSAVIRUS NOMENCLATURE: IS THE RECENT UPDATE OF THE SPECIES NAMES IN THE GENUS LYSSAVIRUS A CONFOUNDING PROBLEM?

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Confusion in the proper use of nomenclature when referring to members of the genus *Lyssavirus* has become increasingly common, particularly, failure to use italics when writing species names and confounding species name with virus names. The relatively rapid evolution of lyssavirus classification due to accelerated discovery of new lyssaviruses, along with advances in virus typing technologies and refinement of phenotypic and phylogenetic approaches are responsible for nomenclature misuse. This communication advocates for correct and harmonized use of nomenclature. In 2015, the ICTV Mononegavirales study group proposed the implementation of taxon-wide not-Latinized binomial species names, including the family Rhabdoviridae. The proposal meant to emphasize the difference between a virus (concrete entity) and its taxonomic classification (abstract concept), but also to more closely reflect the binomial nomenclature used elsewhere in biology in which the species name includes the genus name. Thus, the approved taxonomic denominations for recognized species are; Aravan lyssavirus, Australian bat lyssavirus, Bokeloh bat lyssavirus, Duvenhage lyssavirus, European bat 1 lyssavirus, European bat 2 lyssavirus, Ikoma lyssavirus, Irkut lyssavirus, Khujand lyssavirus, Lagos bat lyssavirus, Mokola lyssavirus, Rabies lyssavirus, Shimoni bat lyssavirus and West Caucasian bat lyssavirus. This new taxonomic designation, does not alter the names of viruses and their respective abbreviations: Aravan virus (ARAV), Australian bat lyssavirus (ABLV), Bokeloh bat lyssavirus (BBLV), Duvenhage virus (DUVV), European bat lyssavirus 1 (EBLV-1), European bat lyssavirus 2 (EBLV-2), Ikoma lyssavirus (IKOV), Irkut virus (IRKV), Khujand virus (KHUV), Lagos bat virus (LBV), Mokola virus (MOKV), rabies virus (RABV), Shimoni bat virus (SHIBV) and West Caucasian bat virus (WCBV). Note that all species names are italicized, start with a capital letter and should never be abbreviated. Conversely, virus names should be capitalized if they include proper nouns (such as names of cities, regions or continents), never be italicized and abbreviations of their names are allowed.



CANADA

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INTERNATIONAL CONFERENCE
ON RABIES IN THE AMERICAS

RABIES CONTROL/CONTRÔLE DE LA RAGE/CONTROL DE LA RABIA/CONTROL DA RAIVA

POSTER PRESENTATIONS/PRÉSENTATIONS
D'AFFICHES/PRESENTACIONES DE
CARTELES/APRESENTAÇÕES DE PÔSTERES

A COMMUNITY BASED KNOWLEDGE, ATTITUDE, AND PRACTICE SURVEY REGARDING RABIES AMONG CATTLE OWNERS IN TWO DISTRICTS (RABIES ENDEMIC AND NON-ENDEMIC) OF BHUTAN

Sangay Rinchen, David Hall, Susan Cork

Presenter: Sangay Rinchen

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Rabies remains a disease of public health and economic concern in Bhutan. The government implements regular mass dog sterilization, anti-rabies vaccination of dogs, and rabies awareness programs as a measure to control rabies and the growing stray dog population. As part of an approach to prevent rabies in cattle in Bhutan, we conducted a community based Knowledge, Attitude, and Practice survey in two districts (rabies endemic and rabies non-endemic) of Bhutan. Five hundred and sixty-three cattle owners were interviewed using a structured questionnaire. The objective of the study was to assess and identify gaps in Knowledge, Attitude, and Practices regarding rabies among the cattle owners to enable informed planning of intervention measures. Eighty-eight percent of the total respondents (n=563) had heard about rabies. In the rabies endemic area, 89% of the respondents (251/281) had heard about rabies whereas in the rabies non-endemic area, 86% of the respondents (243/282) had heard about rabies (difference was not significant at 5% significance level). Male respondents and those having school-going children were more likely to have heard about rabies ($p < 0.01$). Twenty-two percent of the respondents who had heard of rabies did not know the signs shown by a rabid dog while 51% said they would not be able to identify rabies in cattle. Forty-six percent of the respondents owned dogs of which 70% were vaccinated. Based on our knowledge scoring criteria for rabies, of the 494 respondents who had heard about rabies, only 44.5% were observed to have adequate knowledge consistent with prevention and response activities. The percentage of respondents having adequate knowledge in the rabies endemic area was 69%, compared to 20% in the rabies non-endemic area. Our study underlines the need to strengthen health education for rabies prevention across communities.

TARGETING CANINE RABIES IN SIERRA LEONE: A SYSTEMATIC REVIEW

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The 2014 Ebola epidemic and the eleven-year civil war has left Sierra Leone with one of the densest stray dog populations in Africa. Rapid urbanization, conflict, and underdeveloped infrastructure create a high-risk environment for the transmission of zoonotic diseases. Sierra Leone has one of the highest estimated rates of rabies mortality in the world, with over three deaths per 100,000 people annually. While rabies is a preventable disease, vaccination can cost up to \$40 USD in a country where the average income is around \$1 a day. Our objective was to systematically review research articles on dog rabies control in terms of dog demography, type of strategy, and cost of preventive program. Here, we highlight current approaches, as well as some of the perceived barriers for the country in implementing rabies control and elimination programs. The literature review indicated that Sierra Leone is afflicted with epidemiological constraints, operational handicaps, and limited resources for the implementation of rabies surveillance. From these studies we concluded that effective control measures need to center on mass vaccination requiring 70% coverage, restriction of dog movement, dog licensing, and control of stray dogs. By using a One Health model, it is hoped that canine-transmitted rabies will be accorded high priority as a zoonotic disease by international donor agencies and government authorities. Other interventions such as Animal Health Clubs also have the potential to be effective in promoting awareness and generating equitable benefits for human health, particularly in rural areas. Well-designed control programs should include significant animal welfare components aimed at the elimination of pain and suffering, improved rates of survival, and the avoidance of inhumane culling. Foreign aid vaccination programs in conjunction with local educational programs and an accurate surveillance system, constitute a pragmatic way forward for rabies control in Sierra Leone.

INTERVENTIONS FOR RABIES CONTROL OUTBREAKS BY THE NATIONAL PROGRAMME OF ZONOSSES CONTROL AND RABIES NATIONAL CENTER IN PARAGUAY (2014-2017)

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Rabies is a zoonotic viral disease, causes 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 2014 to 2017 in Paraguay. There were notified by SENACSA, 121 cases of animal's rabies (108 cattle, 3 dogs, 1 cat, 3 horses, 1 sheep and 5 vampire bat) from all the 17th departments of the country. There were vaccinated with the Verorab[®] Sanofis[®], France, vaccine against rabies in humans, 580 persons that were exposed to the virus (farmers, animal's owners), and 32943 dogs and cats living in a ratio of 5 kilometers around the animals 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.

EVALUATION OF A CANINE RABIES VACCINATION CAMPAIGN IN THE SOUTH OF GUATEMALA

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Canine rabies is endemic in Guatemala, and between 2005 and 2017, the country reported 956 cases in animals and 18 cases in humans. Annually massive canine vaccinations are carried out, by the Ministry of Health, but they do not have an evaluation system to determinate the real coverage of these campaigns. To help to improve the canine rabies control and eradication in Guatemala, we conducted pilot surveys during the rural national canine rabies vaccination campaign, to evaluate the vaccination coverage in four communities in the south of the country. For these surveys we marked the vaccinated animals using blue collars or blue paint markers, to estimate the vaccination coverage and the dog population in the study area using rapid community dog assessment and sight re-sight surveys. We compared our dog population estimation, and vaccination coverage with the official data reported by the Ministry of Health, and the official canine population was underestimated, and consequently the vaccination coverage was overestimated (70% vs 44%). As a follow up to determinate the vaccination barriers, we conducted a door-to-door survey in the study area after the end of the campaign, and 40% of the respondents reports they did not know that there had been a campaign in their community. This evidenced the lack of communication, advertising and disclosure of the campaign. The results were reported and discussed with the national zoonosis program, which is on charge of the vaccination campaign, and now they are taking actions to stablish a better system to estimate canine population, create a way to do sustained evaluation after the campaign ends, and to improve the publicity and communication for the next campaign.

REALIZACIONES, DESAFÍOS Y PERSPECTIVAS PARA LA ELIMINACIÓN DE LA RABIA TRANSMITIDA POR PERRO EN HAITÍ, 2015-2017

Natael Fenelon, Cerpa Mauricio, Marco A. N. Vigilato² Felipe Rocha² Alexandra Vokaty, Julio A. Pompei, Baldomero Molina Flores, Ottorino Cosivi, Carla Perdiz, Joceline Pierre Louis, Marie Dieuta Joseph

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Introducción: La región de las Américas está muy cerca de la eliminación de la rabia humana transmitida por el perro y Haití es uno de los países prioritarios donde la OPS/OMS, a través de su centro especializado en Salud Pública Veterinaria PANAFTOSA/SPV, trabaja intensamente apoyando las acciones nacionales encaminadas a la eliminación de esta enfermedad.

Metodología: El plan de acción de la 15 REDIPRA ha sido utilizado como guía para la realización de las actividades. Fortalecimiento de la vigilancia epidemiológica de las mordeduras por animales sospechosos. Planificación y realización de capacitación para profesionales de la salud. Investigación de focos rábicos tanto humanos como animales. Apoyo a la descentralización de la vacuna para humanos a través del país. Desarrollo y distribución de materiales educativas para la población. Elaboración de materiales educativas de atención médica para profesionales de salud (algoritmo y guía). Apoyo técnico a la campaña de vacunación de perros.

Resultados: Fueron capacitados 459 profesionales de salud en 140 centros de salud en 8 departamentos los cuales disponen de la vacuna antirrábica y profesionales entrenados. Antes de 2016, solamente 15 centros de salud aplicaban la vacuna antirrábica. 15,000 de dosis de vacuna disponible para las personas expuestas. Elaboración de un algoritmo y una guía de atención médica de las mordeduras que fueron distribuidos en los centros de salud. Para el seguimiento del paciente, una carné de vacunación ha sido creada. Se ha realizado la conmemoración del día mundial de la rabia acompañada de sesiones de sensibilización a la población y vacunación de perros. Se ha iniciado la campaña nacional de vacunación de perros, la última campaña fue en noviembre 2014. En 2017, 51222 perros vacunados aproximadamente.

Conclusión: La eliminación de la rabia humana transmitida por perro es posible mediante el acceso al tratamiento post exposición, la vacunación del 70-80% de los perros anualmente y el fortalecimiento de la información y sensibilización de la población.

ONE HEALTH APPROACH TOWARDS RABIES OUTBREAK CONTROL IN BHUTAN: PREVENTION IS BETTER THAN CURE

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Rabies is a cross border transmission problem in Bhutan. Multiple incursions into interior rabies-free areas have taken place in the recent past, indicating possible re-emergence of rabies in the country. The most recent incursion and outbreak (July 2016 through June 2017) had occurred in eastern Bhutan that shares border with India. On 28 July 2016, a rabies suspected dog became aggressive and had bitten several other free-roaming dogs, which was later confirmed as rabid. Following this incident, an outbreak had spread widely to nearby villages and towns causing death of 37 domestic dogs, 11 cattle and 1 cat. Twenty five people were bitten by confirmed rabid dogs and cat with category III exposure. Several people in the outbreak areas were also indirectly exposed to rabid animals. A Rapid Response Team (RRT) was activated immediately to implement the control measures and typically involves One Health multisectoral approach. The RRT implemented control measures such as: trace-capture and elimination of suspected and confirmed rabid dogs, zoo-sanitary measures; an emergency vaccination of susceptible dogs and cats; surveillance and monitoring; public awareness education, contact-tracing of bite victims and animals, and knowledge and attitude survey. All the bite victims were given post exposure treatment including wound washing with soap and water/antiseptic dressing; infiltration of human rabies immunoglobulin (HRIGs) around the bite site and intradermal rabies vaccination. No human rabies case was reported during this outbreak. Although challenging, the outbreak was contained using One Health multisectoral approach by the RRT. The cost of control program by Bhutanese standard was huge and therefore, it is cost effective to prevent through mass dog vaccination than control outbreak. The lessons learned from this outbreak are many and will be useful for guiding containment activities in any future outbreak in the country and elsewhere in the world.

ONE HEALTH, RABIES PREVENTION AND MORE-THAN-HUMAN CONSIDERATIONS IN INDIGENOUS COMMUNITIES IN NORTHERN AUSTRALIA

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Australia is currently rabies free; therefore, the spread of rabies in eastern Indonesia poses a risk to northern Australia. Dogs are numerous in East Arnhem Land (EAL) and the Northern Peninsular Area (NPA), usually unrestrained and living in close human-dog relationships. The response to any rabies outbreak on Australian territory will focus on dog vaccination, controlling dog movements and depopulation. A One Health approach to zoonotic disease control should seek to co-promote human and animal health, whilst also seeking to accommodate the preferences of affected communities. We report on 4 community panels/workshops and >40 semi-structured interviews conducted with: (i) EAL and NPA community members; (ii) Indigenous Rangers in EAL and NPA; and (iii) residents of Cairns, the local regional centre. We used storyboard methodologies to work with participants to explore the following questions:

1. What do people who live in EAL, the NPA and Cairns think should and should not be done if a rabies incursion was to occur?
2. How should the interests of individuals, communities, and nonhuman animals be accommodated in such a response?
3. What are the roles and responsibilities of dog owners and other community members in the event of a rabies incursion?

The results of this study can inform control strategies that are consistent with community values. We found that the capacity of community members in the NPA and EAL to contribute/adapt to a bio-security response is likely to be limited by material disadvantage, dominant cultural norms and food security concerns. Responsible ownership means different things within and across the study settings; the cultural value placed on dogs/dingoes is variable and conditional. Adopting a 'strengths-based' approach mandates that the communities at greatest risk need to be helped to prepare for and develop strategies to manage a bio-security response to a rabies incursion.

DOG-BITES, RABIES AND ONE HEALTH: TOWARDS IMPROVED COORDINATION IN RESEARCH, POLICY AND PRACTICE

Melanie Rock, Dawn Rault, Chris Degeling

Presenter: Dawn Rault

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Dog-bites and rabies are neglected problems worldwide, notwithstanding recent efforts to raise awareness and to consolidate preventive action. As problems, dog-bites and rabies are entangled with one another, and both align with the concept of One Health. This concept emphasizes interdependence between humans and non-human species in complex socio-ecological systems. Despite intuitive appeal, One Health applications and critiques remain under-developed with respect to social science and social justice. In this article, we report on an ethnographic case-study of policies on dog bites and rabies, with a focus on Calgary, Alberta, Canada, which is widely recognized as a leader in animal-control policies. The fieldwork took place between 2013 and 2016. Our analysis suggests that current policies on rabies prevention may come at the expense of a 'bigger picture' for One Health. In that 'bigger picture,' support is needed to enhance coordination between animal-control and public-health policies. Such coordination has direct relevance for the well-being of children, not least Indigenous children.

THE PAN AFRICAN RABIES CONTROL NETWORK

Louis Nel, Andre Coetzer and Terence Scott * University of Pretoria and the Global Alliance for Rabies Control *on behalf of the members of PARACON

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Following on from the successes of the inaugural Pan African Rabies Control Network (PARACON) meeting in 2015, PARACON has hosted a sub-regional workshop for Francophone countries in West and Central Africa in 2016. This year, we hosted the second sub-Regional PARACON Workshop focused towards Anglophone countries of southern and eastern Africa. The meeting took place in Pretoria, South Africa, 13-15 September 2017.

The sub-regional PARACON workshops are relatively small working meetings, with tangible outputs. The active participation of each delegate was largely focused on the newly revised Stepwise Approach towards Rabies Elimination (SARE) tool (and all the associated tools embedded in the rabies blueprint) as well as the PARACON Epidemiological Bulletin.

We aim to provide a comprehensive feedback of the outcomes of this meeting. Among others we will discuss the uptake and impact of the various tools, and their roles in national and regional planning strategies as we work together towards the 2030 rabies elimination goal.

ANALISIS HISTORICO DE LA RABIA EN EL ESTADO DE NUEVO LEON, MEXICO

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A nivel mundial la rabia se presenta en aproximadamente en 150 países y en el continente Americano se tiene un registro de casos de rabia humana entre 5-15 por año. En México se reporta un promedio de 2.8 casos de rabia humana por año en los últimos 17 años en 15 estados. En el estado de Nuevo León se cuenta con el último caso de rabia humana en 1986 transmitido por perro y 1988 transmitido por murciélago. A partir de estas fechas se realizan campañas masivas para el control de rabia canina, la cual el último caso fue en 1995, reapareciendo en el 2010 y teniendo el último registro de un canino en el 2011. Se demuestran las acciones de control y prevención realizadas por la Secretaria de Salud del estado de Nuevo León limitar la reintroducción de rabia humana al estado. En los últimos años, el esfuerzo entre el estado y nivel federal ha ido generando un aumento en el control y prevención de la enfermedad y en los últimos 7 años se han tratado a 5,234 personas por estar en riesgo, se han vacunado 6, 909,298 mascotas, se han esterilizado 104,241 mascotas. Se han examinado 23,728 muestras de mascotas y fauna silvestre teniendo una positividad de solo 20 muestras (0.08%). En años recientes se ha incrementado la positividad en muestras proveniente de fauna silvestre siendo el grupo de los murciélagos insectívoros los que reportan un mayor número de casos positivos. Ello implica que el esfuerzo de la Secretaria de Salud y la ciudadanía se deben enfocar a fortalecer el desarrollo de acciones de vigilancia, prevención y control para evitar la transmisión a los humanos.

SPATIAL HETEROGENEITY IN VACCINE COVERAGE BETWEEN FIXED POINT VS MOBILE TEAM APPROACHES IN AREQUIPA, PERU

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In March 2015 rabid dogs were detected in Arequipa, Peru, signaling the reintroduction of the virus in this area. Since then, numerous efforts have been made to control the outbreak, including multiple mass dog vaccination campaigns, however rabid dogs have been detected almost every month until the present. The approaches for dog vaccination campaigns in the city have varied even within the same district: in some areas mobile teams were deployed while in others fixed points were used. However, high spatial heterogeneity of vaccination coverage can reduce the likelihood of successful rabies elimination. In collaboration with the Ministry of Health, we conducted longitudinal door-to-door surveys over two consecutive years in urban and peri-urban communities of Arequipa to assess the spatial heterogeneity in vaccination coverage resulting from these two approaches: mobile team vs fixed point. Overall, we found lower spatial heterogeneity of vaccination coverage in areas served by fixed points. However, we also found that for households located more than 500m from the fixed-point vaccination, participation in the campaign reduced greatly. On the contrary, areas served by mobile teams showed “patchy” coverage (higher heterogeneity). In Arequipa, a large city with more than 1 million people, the most important indicator of dog vaccination success is the WHO benchmark of 70% overall coverage. However, as our results indicate, coverage varies by district and varies greatly at the sub-district level depending on the vaccination approaches implemented in the field. Highly heterogeneous coverage creates pockets of unvaccinated dogs that could allow for the persistence of the rabies virus transmission in this area of Peru. Institutional Review Board approval was obtained for both the qualitative and the door-to-door survey from Universidad Peruana Cayetano Heredia, Tulane University, and University of Pennsylvania.

IMPLEMENTACIÓN DEL PLAN ANDINO DE ELIMINACIÓN DE RABIA HUMANA TRANSMITIDA POR PERRO Y DISMINUCIÓN DE LA RABIA TRANSMITIDA POR ANIMALES SILVESTRES - RESULTADOS COLOMBIA 2016

Angela Xiomara Castro Duran

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En Colombia, a medida que se ha disminuido la presentación de casos de rabia humana transmitida por perros, se ha evidenciado un incremento en la presentación de casos producidos por rabia silvestre en casi todas las regiones del país. Para los años 2015, 2016 y 2017 se presentaron tres casos de muerte por rabia en humano provenientes del departamento de Cundinamarca. Existen múltiples factores de riesgo que incrementan el riesgo de infección por este virus en el territorio nacional, que hacen referencia a la circulación del virus rábico en las distintas zonas del país, primordialmente boscosas y selváticas, en donde habitan murciélagos y zorros, vectores importantes del virus, así como la intermediación de los gatos, que facilitan el paso del virus rábico silvestre a los demás animales e individuos de su entorno. Teniendo en cuenta la problemática durante la reunión de directores de rabia en la Américas –REDIPRA-, se realizó una Comisión Técnica que elaboro la necesidad de la intervención en estos países, documento que se presento en la reunión de ministros del área andina, en donde se aprobó la resolución XXXV/497 enfocada a desarrollar un Plan Andino para la Eliminación de la Rabia. Por lo anterior, se realizó la reunión en la ciudad de Arica – Chile, consolidando la situación de la rabia del área andina y planteando líneas de trabajo a desarrollar durante el año 2016. Durante el año 2016, El Ministerio de Salud de Colombia implementó dicho plan en los departamentos de frontera con el país de Venezuela, desarrollando los 7 puntos propuestos, obteniendo los siguientes Resultados:

1. Garantía del acceso oportuno y la disponibilidad y calidad de agentes inmunobiológicos para las personas expuestas al virus de la rabia
2. Mantenimiento de la cobertura de vacunación adecuada de los perros en las zonas de riesgo
3. Fortalecimiento del plan de acción nacional para el control de la rabia
4. Fortalecimiento del sistema de vigilancia epidemiológica de la rabia humana transmitida por perro
5. Apoyo a la red nacional de laboratorios de diagnóstico de rabia
6. Implementación de mecanismos de educación y comunicación sobre el riesgo
7. Implementación de acciones preventivas frente a la rabia humana de origen silvestre transmitida por murciélagos hematófagos

FERAL AND DOMESTIC CAT VACCINATION IN ORDER TO CONTROL HUMAN RABIES & RABIES VACCINES MUST BE PROVIDED AT LOW COST TO THE VETERINARIAN PROFESSIONAL IN LATIN AMERICAN COUNTRIES

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Most cases of Human Rabies in Colombia and many other Latin American countries are due to bites by infected cats. The cats (domestic and feral) are being infected by V3 and V4 variants transmitted by haematophagous and insectivorous bats. But most of the vaccination campaigns are focused in dogs. Even the OIE posters and mimes have always a dog on it..It is time we do focus in mass immunization for feral and domestic cats in Latin America. This could be accomplished for example with the use of recombinant baited rabies vaccine, as it was done in the late 80's in Europe to control Rabies in Foxes and since late 90's in USA in Coyotes and Raccoons. In USA it is also a clear the link in the number of raccoon rabies reports and domestic cat rabies reports. Another point is that we must ensure the Veterinarian Professional and all staff involved in mass vaccination campaigns, gets vaccinated against rabies at a minimal cost . In many Latin American countries the price of the human rabies vaccine is prohibitive. What they would do?, immunize themselves with cat & dog Rabies vaccine products they do have in their Veterinary Clinics then?. We must address these 2 issues if in fact we want to reach the 0 cases of Human rabies by 2030 Global goal.



**EPIDEMIOLOGY AND SURVEILLANCE/ÉPIDÉMIOLOGIE ET
SURVEILLANCE/EPIDEMIOLOGÍA Y
VIGILANCIA/EPIDEMIOLOGIA E VIGILÂNCIA**

**POSTER PRESENTATIONS/PRÉSENTATIONS
D'AFFICHES/PRESENTACIONES DE
CARTELES/APRESENTAÇÕES DE PÔSTERES**

SUVIS AND YOU ... KNOWING THE ACTIONS OF SUVIS SÃO MIGUEL IN ITS TERRITORY, VIEWING IMPROVEMENT IN THE PREVENTION OF THE TRANSMISSION OF HUMAN RABIES (SUVIS E VOCÊ... CONHECENDO AS AÇÕES DA SUVIS SÃO MIGUEL NO SEU TERRITÓRIO, VISANDO MELHORIA NA PREVENÇÃO DA TRANSMISSÃO DA RAIVA HUMANA)

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

Presenter: João Gabriel Zerba Corrêa

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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.

RABIES IN CANADA – 2016

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In 2016, the CFIA laboratories tested 3057 samples for rabies, of which 392 (12.8%) were positive. Of these, 259 were confirmatory tests on wildlife surveillance samples, with no known human or animal contacts, which were initially analysed in provincial laboratories. At the CFIA, the majority of samples were analysed by the fluorescent antibody test, with a small number tested by immunohistochemistry (n=2) or quantitative RT-PCR (n=6, human suspect cases, all negative). There was a significant increase in case detection from 2015 (6.6% positive), attributable to an ongoing outbreak of raccoon-variant rabies in southwestern Ontario first detected in December 2015 (258 cases in 2016). Not surprisingly, given this outbreak, raccoons accounted for the highest proportion of cases in 2016 (44%), followed by skunks (29%) and bats (20%). Spill-over of wildlife virus variants into domestic animals was observed, with cases detected in dogs (n=2), bovines (n=5), cats (n=4), goats (n=2), one horse, one llama, and one sheep, the result of either skunk-variant virus in western Canada (n=13) or in southwestern Ontario either the fox-variant virus (n=1) or raccoon-variant virus (n=2). In regions north of 55°, no spillover of fox rabies into domestic dogs was detected for the first time since 2010. A fox-variant virus outbreak first detected in Labrador in 2014 ended in 2015 with no new cases reported in 2016. The 2014 incursion of rabies caused by the raccoon-variant virus in New Brunswick continued, but only 3 cases were detected each in 2016 and the first half of 2017. In contrast, the raccoon rabies outbreak in southwestern Ontario has been much more extensive, with 342 cases detected between December 2015 and June 30 2017. Interestingly, unlike previous incursions of raccoon rabies in the US, relatively few cases of spillover into domestic animals have been observed (2 cats, 1 llama).

MORE THAN JUST SKUNKS! RABIES IN MANITOBA

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Issue – Since April 2014, Manitoba Rabies Central (MRC) has operated the Provincial Rabies Management Program. MRC represents the departments of Manitoba Health, Seniors and Active Living (MHSAL); Manitoba Agriculture (MB Ag); and Sustainable Development (SD). Several trends can be identified from the sample collection data since that time.

Setting – Manitoba has a high burden of rabies, particularly in the Southern half of the province where the skunk variant is endemic in the striped skunk population. In Northern Manitoba, the Arctic fox variant predominates and the Arctic fox is the primary reservoir.

Results – In the first two years of the program, 13% of 275 samples tested were positive. Most positive samples were skunks (25), followed by cattle (5); the most frequently sampled species were dogs, cats and skunks. All positive samples were from the Southern half of Manitoba.

Implications – During the first 2 years of the program:

1. Samples collected from some rural Health Regions increased significantly from year 1 to 2. This may have been due in part to geographical clustering of some positive samples, leading to increased community awareness and reporting of possible exposures.
3. Decreases in the number of dogs tested suggest increasingly effective and scientific risk assessments are being conducted for potential human exposures to rabies from dog bites.
4. A minimal number of sample testing requests, despite a high number of reports of suspected rabies exposures, from the Northern Health Region were noted. Regional Public Health staff identified that significant community outreach, along with dedicated animal control staff, have led to animals being quarantined in almost all exposures.
5. Significantly more samples were collected from cattle in year 2 than year 1. Exposure events occurred in known high prevalence areas for rabies in skunks and high density of the province's beef cattle herd.

SURVEILLANCE OF RABIES IN WILD ANIMALS IN RUSSIA

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The Russian Federation permanently affected with rabies. Annually from 2 to 4 thousand cases of rabies in animals are recorded. In the RF, rabies is a disease primarily of wild carnivores, which are the main reservoir and vector of rabies. The incidence of rabies among wild animals in 2016 was 1,124 cases (52% of the total). This is 25% lower than the average for the last 5 years (1,493 cases), and almost a twofold decrease in the incidence (minus 47%) compared to 2015 (1,974 cases). Among wild animals in 2016, in 70% (777 cases) rabies was detected in foxes (*Vulpes vulpes*), 20% (222 cases) - in raccoon dogs (*Nyctereutes procyonoides*). Other wildlife species have no more than 2% of the total number of cases and the important role in the epizootic process did not play. In 2016, fox's rabies was registered in 48 subjects of the RF. The highest incidence rates of rabies in foxes were recorded in Moscow (121 cases - 16%), Yaroslavl (52 - 7%), Kirov (49 - 6%) regions, and the Republic of Udmurtia (61 - 8%). Raccoon dog's rabies was registered in 24 subjects of the RF. The species has a smaller habitat on the RF. The greatest number of diseased animals was identified in Tver (47 cases, 21%), Yaroslavl (39, 18%) and Moscow (30, 14%) regions. Rabies in other wildlife species was registered in 89 cases (8% of wildlife): Carnivorous/Carnivora – 58 cases, Rodents/Rodentia – 14, Artiodactyls/Artiodactyla – 9, Insectivorous/Eulipotyphla 7, Lagomorpha/Lagomorpha -1. In conclusion, the dynamics of the epizootic process of rabies in wildlife in RF has a wave character. In 2016, we observed a decline of indicators: decreased incidence of rabies, reduce the area of affected regions. In addition, a positive effect has on going vaccination of wild animals in many regions of the Russian Federation.

EPIDEMIOLOGICAL SITUATION OF RABIES IN PARAGUAY (2014-2017)

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Rabies is a zoonotic disease caused by viruses from the genus *Lyssavirus* associated with neurological signs because of the development of encephalitis or meningoencephalitis. In Paraguay, rabies is one of the causes of large economic losses in the productive sector (animal deaths) as well as in the public sector due to the elevated costs of monitoring and control programs. 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*), on strategic vaccination and active/passive epidemiological surveillance in humans, dogs and cats. The aim of this study was to describe the epidemiological situation of rabies in Paraguay. It was carried out by using data from the government agency responsible for animal health in Paraguay (SENACSA and PNCZ y CAN), which covers diagnosis from animals suspected of rabies carried out between 2014-2017. The samples came from all regions of the country and were sent voluntarily by farmers or by both private and public veterinarians. The samples sent to the government animal health laboratory were subjected to the direct immunofluorescence technique, rabies virus typification by indirect immunofluorescence monoclonal antibodies technique and to the biological proof (inoculation in mice). From 2014 to 2017, 1552 samples of animal's brains were

analyzed from all the 17th departments of the country. Positive rabies cases were detected in 108 cattle, 3 dogs, 3 horses, 1 cat, 1 sheep and 5 vampire bats. The antigenic variants diagnosed were 2, 3 and 4. Rabies in Paraguay is part of a complex situation and further research is needed. Several socioeconomic and environmental factors must be taken into account. Nevertheless, the method applied in this study allowed us to establish priorities for epidemiologic surveillance.

REEMERGENCE OF RABIES VIRUS MAINTAINED BY CANID POPULATIONS IN PARAGUAY

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Paraguay has registered no human cases of rabies since 2004 and the last case in dogs, reported in 2009, was due to a variant maintained in “Desmodus rotundus”. In 2014, a dog from the “Chaco” region was diagnosed as positive for rabies with aggression towards a boy. Epidemiological investigation revealed that the dog was not vaccinated and was likely attacked by a crab-eating fox (*Cerdocyon thous*). In 2015, a second case from the same district was diagnosed in a dog, without reports of aggression by *C. thous*. These samples, and eleven samples from an outbreak in Assuncion in 1996, were submitted to antigenic and genetic characterization. Antigenic characterization showed that the samples presented the antigenic profile, AgV2, related to the variant maintained by dogs in Latin America. For the genetic characterization, the phylogenetic tree was reconstructed with sequences of samples from this study and representative sequences isolated in the Americas. The samples from 2014 and 2015 segregated in the canine (domestic and wild species) related group in a subgroup that also included samples from a dog and a crab-eating fox from the Chaco region of Argentina. The samples from the 1996 outbreak segregated in the canine group with four samples from other regions of Argentina. These results are indicative that rabies virus is circulating in canid populations between these two countries, particularly in the “Chaco” region. The epidemiology of the 2014 case indicates that despite being under control in domestic dogs, rabies virus can be circulating in the crab-eating fox population; as observed in northeast Brazil, where wild dogs maintain a virus lineage derived from the virus of domestic dogs. These results demonstrate the importance of maintaining vaccinations in domestic animals, the collaboration between countries for rabies control and continuous surveillance activities to control the disease in wildlife.

ANTIGENIC AND GENETIC CHARACTERIZATION OF RABIES VIRUS STRAINS, CIRCULATING IN THE STATE OF PARÁ, BRAZILIAN AMAZONIA, BETWEEN 2015 AND 2016

Taiana Andrade Freitas, Livia Medeiros Neves Casseb, Taciana Fernandes Souza Barbosa Coelho, Samir Mansour Moraes Casseb, Armando de Souza Pereira, Francisco Hamilton dos Santos Paiva, Érika Dayane Leal Rodrigues, Elizabeth Salb  Travassos da Rosa.

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Understanding the evolution of Rabies lyssavirus (RABV) is important to establish bases of phenotypic and genotypic variability of the viral populations and the emergence of new variants, and mutations that can alter the pathogenicity and virulence of the virus. Molecular biology has made advances, and revealed that although there are antigenic differences in RABV isolates in the most varied species, these isolates have conserved changes in the gene, allowing them to be used as epidemiological markers. Being the antigenic and genetic characterization, important tools in the determination and understanding of multiple epidemiological cycles and the potential of interspecies transmission. The objective of this study was to characterize antigenically and genetically strains of rabies virus isolated in the State of Par , from biological samples (nervous tissue) of animals, received at the Evandro Chagas Institute, between the years 2015 to 2016, coming from different mesoregions of the State, with positive results in the tests of Direct Immunofluorescence and Viral Isolation in Mouse. A total of 29 samples were antigenically characterized by Indirect Immunofluorescence (IFI), using a panel of eight monoclonal antibodies produced by CDC/Atlanta, USA. Of these, six samples that were not conclusive in the IFI were genetically characterized by partial nucleotide sequencing of the N gene, followed by phylogenetic analysis. The sequences obtained were compared to each other and with in RABV sequences already detected in Brazil and in the Americas, using the neighbor-joining method. In the tests used, the antigenic variant 3 (VAg3) of RABV was identified in all samples. It was observed a greater circulation of the virus in the Southeast of Par  (n = 22), followed by Northeast of Par  (n = 5), being the animals of production most affected by the disease. These analyzes enables to understand the epidemiological profile of rabies in the State of Par , emphasizing the importance of VAg3 transmission, commonly associated with *Desmodus rotundus*, which is the most prevalent hematophagous bat in the Americas, and is the main responsible for the transmission of rabies to herbivores.

SCOPING THE EVIDENCE: A REVIEW OF DOG BITE-RELATED SEQUELAE

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OBJECTIVES: The diseases and other sequelae resulting from dog bites have been documented throughout history, starting with rabies. Summarising and charting the research and non-research related material pertaining to dog bites, sequelae, and prevention was deemed significant in providing an overview of the existing relevant information. It was critical to follow the existing evidence available on dog bites and attacks, dog population management, dog bite prevention, and zoonotic pathogen transmission and elimination to impart a holistic picture of dog bite epidemiology and sequelae.

METHODS: This scoping review charted the published peer-reviewed and non-peer-reviewed grey literature and online information to look at the identified outcomes of dog bite episodes. Each consequence was also assessed from the viewpoint of remote Canadian indigenous communities.

SYNTHESIS: Peer-reviewed literature (N=693; review, original research and review articles) provided quite detailed information on specific pathogens, infections and diseases of interest, especially rabies. However, in addition to these, the sequelae from dog bites can include moderate to severe injuries that can further result in anxiety around dogs or post-traumatic stress disorder (PTSD).

CONCLUSIONS: While significant focus has been placed on rabies as a sequela from dog bites, the effects of anxiety and PTSD are not as well described. Although treatment of bite injuries and infections can be normalised, research and development of relationships and prevention education across health disciplines, including counselling services, and between disciplines, such as veterinarians, animal behaviourists and other health professionals, could be a major advantage in decreasing bites and their sequelae.

ACCIONES DE CONTROL DE LA RABIA EN BOVINOS Y ESPECIES GANADERAS EN EL ESTADO DE HIDALGO

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INTRODUCCIÓN

EL ESTADO DE HIDALGO TIENE UNA SUPERFICIE DE 20,987 KM², CON 621,478 CABEZAS DE GANADO BOVINO EN 54,273 HATOS. EL COMITÉ ESTATAL DE FOMENTO Y PROTECCIÓN PECUARIA DEL ESTADO DE HIDALGO REALIZA ACCIONES DE CONTROL DE RABIA EN 59 MUNICIPIOS, DE LOS 89 MUNICIPIOS QUE INTEGRAN EL ESTADO. CLASIFICADOS POR SU NIVEL DE INCIDENCIA A LA ENFERMEDAD EN BAJA, MEDIANA, ALTA Y SIN REPORTE DE ANIMALES CON SIGNOLOGÍA NERVIOSA, NI PRESENCIA DE MURCIÉLAGO HEMATÓFAGO

OBJETIVO

PREVENIR Y CONTROLAR LA PRESENCIA DE CASOS POSITIVOS A RABIA EN EL GANADO, SALVAGUARDANDO LA SALUD PÚBLICA. PROBLEMÁTICA EL 80% DE LOS CASOS POSITIVOS SON EN ANIMALES SIN UN CALENDARIO DE VACUNACIÓN ADECUADO O EN ANIMALES NUNCA VACUNADOS. LA FALTA DE SEGUIMIENTO POR PARTE DE LOS PRODUCTORES A LA ENFERMEDAD, ASÍ COMO LAS MEDIDAS DE PREVENCIÓN Y CONTROL QUE SE PUEDEN ESTABLECER MEDIANTE CAMPAÑAS.

MÉTODOS

SE REALIZAN ACCIONES EPIDEMIOLÓGICAS PARA LA PREVENCIÓN Y CONTROL, COMO SON LA VACUNACIÓN DE GANADO BOVINO Y ESPECIES SUSCEPTIBLES, EL CONTROL DEL MURCIÉLAGO HEMATÓFAGO MEDIANTE LA REALIZACIÓN DE OPERATIVOS DE CAPTURA Y LA IMPARTICIÓN DE ACTUALIZACIONES TÉCNICAS DIRIGIDAS A PRODUCTORES CON EL APOYO DE MATERIAL DE DIFUSIÓN. UN PUNTO IMPORTANTE QUE FORTALECEN LAS ESTRATEGIAS EPIDEMIOLÓGICAS ES LA REALIZACIÓN ES LA CAPACITACIÓN Y DIFUSIÓN MEDIANTE CONCURSOS DE DIBUJO INFANTIL EN ESCUELAS PRIMARIAS, DISTRIBUCIÓN DE MATERIAL DE DIFUSIÓN, CONVENIOS CON PRESIDENCIAS MUNICIPALES, SECTORES PÚBLICOS Y PRIVADOS REALIZANDO ACCIONES EN CONJUNTO, COMO LO SON LA ROTULACIÓN DE BARDAS, SPOTS DE RADIO, MATERIAL IMPRESO, CALCOMANÍAS, IMPLEMENTACIÓN DE BANCOS DE VACUNA, ENTRE OTROS.

RESULTADOS

GRACIAS A LOS RECURSOS OBTENIDOS POR LOS GOBIERNOS FEDERALES Y ESTATALES, SE PUEDEN IMPLEMENTAR CAMPAÑAS BAJO ESTRATEGIAS EPIDEMIOLÓGICAS APROVECHANDO LOS RECURSOS ASIGNADOS PREVINIENDO LA ENFERMEDAD.

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