

El Centro de Investigaciones Regionales “Dr. Hideyo Noguchi” de la Universidad Autónoma de Yucatán

invita al

First Trop-G meeting / Primera Reunión Trop-G

Tropical Medicine in the Gulf of Mexico/Medicina Tropical en el Golfo de México

November 4-5, 2015

Edificio Central UADY, Sala del Consejo Universitario
Calle 60 #491-A x 57, Mérida, Centro, Yucatán

Entrada libre



Tropical Medicine in the Gulf of Mexico, Trop-G, is a collaboration of Baylor College of Medicine, Tulane University, and Universidad Autónoma de Yucatán that focuses on Neglected Tropical Diseases (NTDs) in the Gulf Coast states of Mexico and the United States (US). Trop-G is an international consortium with the aim of controlling and eliminating NTDs from this high-risk area (<http://trop-g.org/>).

This first meeting will provide a forum for interactions among scientists, health professionals, and government officials from institutions around the Gulf of Mexico, to expand collaborations and work towards an improved control of NTDs in the area.

The event includes scientific presentations, round tables and poster sessions, to provide opportunities for discussions and exchange.

Medicina Tropical en el Golfo de México, Trop-G, es una colaboración de Baylor College of Medicine, Tulane University, y Universidad Autónoma de Yucatán y se enfoca en las Enfermedades Tropicales Desatendidas (ETDs) en los estados de la Costa del Golfo de México y de los Estados Unidos (EEUU). Trop-G es un consorcio internacional con la meta de controlar y eliminar las ETDs de esta región de alto riesgo (<http://trop-g.org/>).

Esta primera reunión proporcionará un foro para interacciones entre científicos, profesionales de la salud y gobiernos de instituciones alrededor del golfo de México, para fomentar colaboraciones y trabajar para mejorar el control de las ETDs en la región.

El evento incluye presentaciones científicas, mesas redondas y sesiones de carteles, para proporcionar oportunidades de discusión e intercambio.

Program / Programa

November 4, 2015

- 5:00 pm - 6:00 pm Inauguration of the Unit for Animal Experimentation (UEA), Health Science Campus (Inalambrica Building)
Inauguración de la Unidad de Experimentación Animal (UEA), Campus de Ciencias de la Salud (edificio inalámbrica)
- 6:00 pm - 7:00 pm Opening ceremony, Central Building UADY, Hall of the University Council
Inauguración del evento, Edificio Central de la UADY, Salón del Consejo Universitario
- 7:00 pm - 9:00 pm Welcome cocktail
Coctel de bienvenida

November 5, 2015

- 8:00 am - 9:00 am Registro/Registration

Opening lectures

- 9:00 am - 9:35 am Peter Hotez, Baylor College of Medicine, Houston, Texas
"Neglected diseases in the Americas: A new generation of antipoverty vaccines"
- 9:35 am - 10:10 am Pierre Buekens, Tulane University, New Orleans, Louisiana
"Global health and tropical medicine"
- 10:10 am - 10:25 am Break

Symposium 1. Infectious disease epidemiology and control

- 10:25 am - 10:40 am Kristy Murray, Baylor College of Medicine, Houston, Texas
"Emerging Vector-borne and Zoonotic Diseases: From Texas to Nicaragua"
- 10:40 am - 10:55 am Job Lopez, Baylor College of Medicine, Houston, Texas
"Understanding the ecology and pathogenesis of tick-borne relapsing fever spirochetes"
- 10:55 am - 11:10 am Dawn Wesson, Tulane University, New Orleans, Louisiana
"Evaluation of Lethal Ovitrap for Dengue Control in Peru"
- 11:10 am - 11:25 am Jorge Zavala, Universidad Autónoma de Yucatán, Mérida, Yucatán
"Rickettsia in Mexico"
- 11:25 am - 11:40 am Carlos Machain, Universidad Autónoma de Yucatán, Mérida, Yucatán
"History, perspective and opportunities for arbovirology research in the Yucatan Peninsula"
- 11:40 am - 11:55 am Miguel Betancourt, Carlos Slim Health Institute
"Cartilla de vacunación electrónica"



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11:55 am - 1:00 pm **Poster session and coffee**

1:00 pm - 2:30 pm Lunch

Symposium 2: Chagas disease

2:30 pm - 2:45 pm Eric Dumonteil, Universidad Autónoma de Yucatán, Mérida, Yucatán
"Chagas disease control: what do we need?"

2:45 pm - 3:00 pm Jorge Pedro Martin, DNDi Latin America
"DNDi regional strategy for Chagas disease, the path from I+D to access"

3:00 pm - 3:15 pm Claudia Herrera, Tulane University, New Orleans, Louisiana
"*Trypanosoma cruzi* molecular epidemiology in the US: rethinking DTU distribution?"

3:15 pm - 3:30 pm Pierre Buekens, Tulane University, New Orleans, Louisiana
"Congenital Chagas disease"

3:30 pm - 3:45 pm Kathryn Jones, Baylor College of Medicine, Houston, Texas
"Immunogenicity and Efficacy of a Subunit Therapeutic Vaccine Against Chagas Disease"

3:45 pm - 4:00 pm Meagan Barry, Baylor College of Medicine, Houston, Texas
"A therapeutic nanoparticle vaccine against *Trypanosoma cruzi* in a mouse model of Chagas disease"

4:00 pm - 4:15 pm Jonathan Respress, Baylor College of Medicine, Houston, Texas
"Chagasic Cardiomyopathy: Modeling Chronic Disease in Mice"

4:15 pm - 5:15 pm **Poster session and coffee**

Symposium 3. Vaccine development

5:15 pm - 5:30 pm Preston Marx, Tulane University, New Orleans, Louisiana
"Research Resources in the Department of Tropical Medicine"

5:30 pm - 5:45 pm Maria Elena Bottazzi, Baylor College of Medicine, Houston, Texas
"Vaccines against helminth infections: A dream turning into reality"

5:45 pm - 6:00 pm Uli Strych, Baylor College of Medicine, Houston, Texas
"Vaccine Development against Severe Acute Respiratory Syndrome and Middle East Respiratory Syndrome"

6:00 pm - 6:30 pm **Round table**

"Promoting Trop-G and NTD control around the gulf of Mexico: Where do we go from here?"
Peter Hotez, Pierre Buekens, Jorge Zavala

Poster presentations

1. A CANDIDATE THERAPEUTIC VACCINE FOR HUMAN CHAGAS DISEASE: COMPARISON OF IMMUNE RESPONSES TRIGGERED BY DIFFERENT DELIVERY PLATFORMS

J. Pollet¹, K. Jones¹, N. Yoshida², B. Keegan¹, L. Versteeg¹, L. Feng², C.M. Beaumier¹, F. Gusovsky³, P.J. Hotez¹ and M.E. Bottazzi¹

¹ Sabin Vaccine Institute and Texas Children's Hospital Center for Vaccine Development, National School of Tropical Medicine, Baylor College of Medicine Houston, Texas, USA; ² Eisai Inc. RTP, Global Formulation Research, USA

Chagas disease is a neglected tropical disease endemic to Latin America and emerging globally including in the southern United States. The disease is caused by *Trypanosoma cruzi*, an intracellular parasite, which, over time, can migrate to the heart leading to life-threatening Chagasic cardiomyopathy. In the absence of a vaccine, the current first-line treatment option is benznidazole, a chemotherapeutic agent that reduces the parasite burden, but also evokes substantial side effects; it is contraindicated in pregnancy. Our overall aim is to develop a prototype therapeutic vaccine for Chagas disease in humans to delay the onset of Chagasic cardiomyopathy. Our approach includes evaluating this vaccine as both a stand-alone immunotherapy and in combination with benznidazole, and bridging the tolerability and efficacy gap of the drug treatment. Preliminary data has shown that to effectively control *T. cruzi*, a T_H1-mediated CD8⁺ T cell driven immune response is desired. Therefore, we have performed a series of experiments to evaluate formulations of target candidate antigens with the immunostimulatory E6020 adjuvant (a TLR4 agonist, known to elicit strong mucosal and cytotoxic T-cell responses), with respect to induction of T cell immunity, reductions in *T. cruzi* parasite loads and increases in murine host survival. Preclinical immunization studies in mice with a lead vaccine candidate, recombinant Tc24 (modified by site-directed mutagenesis), resulted in an increase of antigen-specific IFN γ -producing CD8⁺ T cells in the spleen. To optimize the vaccine delivery, a comparison of immunogenicity and efficacy of the modified Tc24 using formulations of E6020 entrapped in micelle suspensions, squalene emulsions, and PLGA microparticles was assessed for their ability to induce antigen specific IFN γ , IL-4 and IL-10 from splenocytes, as well as IgG2a antibodies. Squalene emulsions, one of the most effective delivery platforms, were used to compare dose responses. Finally, we present the results of a mouse efficacy study showing that a vaccine containing Tc24 and E6020 in a squalene emulsion can significantly reduce parasitemia levels and increase the survival rate in mice with acute Chagas infection. Future studies will include combination therapy with benznidazole and focus more on the chronic Chagas infection.

Grant funding acknowledgements: Global Health Innovative Technology (GHIT) Fund, Carlos Slim Foundation, Sabin Vaccine Institute and Texas Children's Hospital Center for Vaccine Development and SouthWest Electronic Energy Medical Research Institute.

2. RESPUESTA INMUNE SECUNDARIA A LOS ANTÍGENOS RECOMBINANTES TSA-1 Y Tc24 EN INDIVIDUOS SEROPositIVOS A *TRYPANOSOMA CRUZI* Y ASOCIACIÓN CON SUS SUPERTIPOS DE ALELOS HLA-I.

Liliana E. Villanueva Lizama, Amaru del C. Aguilar Cetina, Vladimir Cruz Chan, Ma. Jesus Ramirez Sierra, Miguel E. Rosado Vallado, Eric Dumonteil.

Laboratorio de Parasitología, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán, Mérida Yucatán, México. Los antígenos rTSA1 y rTc24 fueron utilizados para estimular cultivos de células mononucleares de individuos seropositivos (n=20) y seronegativos (n=19) a *Trypanosoma cruzi*. Se evaluó la proliferación celular, las subpoblaciones de células de memoria y la producción de INF- γ e IL10 intracelular, por citometría de flujo. Se identificaron los alelos de HLA-I de cada participante por PCR-SSP. Se observó proliferación en células CD3⁺ y CD4⁺ de los seropositivos en comparación con seronegativos. Se encontró mayor frecuencia de células T efectoras de memoria específicas de Tc24 en individuos seropositivos en comparación con seronegativos (p<0.05). Las células CD4⁺ estimuladas con Tc24 tuvieron mayor expresión de INF γ e IL10 en comparación con seronegativos. Los alelos HLA-A-24:02:01 (22.7%), HLA-A-68:01:02 (18%), HLA-B-35:01:01 (15.7%) y HLA-B-39:05:01 fueron los más frecuentes en pacientes seropositivos. Se realizará un análisis de correlación para asociar la respuesta inmune celular con los supertipos de alelos encontrados. Los resultados demuestran la presencia de células de memoria de los antígenos TSA1 y Tc24 en pacientes seropositivos que pueden ser activadas *in vitro* para el desarrollo de una respuesta inmune secundaria específica de antígeno. Los resultados apoyan el desarrollo de los antígenos TSA1 y Tc24 como candidatos a vacunas contra la enfermedad de Chagas.

3. DESARROLLO DE UNA METODOLOGÍA PARA EVALUAR LA ACTIVIDAD CITOTÓXICA DE LINFOCITOS T CD8+ ESTIMULADOS CON ANTÍGENOS CANDIDATOS A VACUNA CONTRA *TRYPANOSOMA CRUZI*.

Adriana S. Solís Angulo, Liliana E. Villanueva Lizama, Vladimir Cruz Chan, Ma. Jesús Ramírez Sierra, Miguel E. Rosado Vallado, Eric Dumonteil.

Laboratorio de Parasitología, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán. No se dispone de medicamentos eficaces ni vacunas contra la enfermedad de Chagas, por lo que se ha propuesto desarrollar una vacuna inmunoterapéutica basada en los antígenos recombinantes TSA1 y Tc24, como alternativa al tratamiento de pacientes con enfermedad de Chagas crónica. Estos antígenos han demostrado inducir protección en modelos animales y recientemente se evaluó su inmunogenicidad en células mononucleares de individuos seropositivos a *T. cruzi* demostrando la presencia de células

de memoria específicas de estos antígenos en humanos. Se sabe que la activación de linfocitos T CD4⁺ y CD8⁺ es necesaria para el control de *T. cruzi*. Particularmente, los linfocitos T CD8⁺ poseen mecanismos de citotoxicidad que eliminan células infectadas. En este contexto, proponemos desarrollar una metodología para evaluar la eliminación *in vitro* de células infectadas, lo que permitirá evaluar la citotoxicidad de linfocitos T CD8⁺ estimulados con antígenos candidatos a vacuna contra la enfermedad de Chagas. Esta metodología utilizará tripomastigotes de *T. cruzi* marcados con el fluorocromo V450 lo que permitirá evaluar el porcentaje de infección y eliminación de parásitos en cocultivos de linfocitos y células dendríticas autólogas, utilizando citometría de flujo. Los resultados proporcionarán una herramienta para la evaluación de vacunas contra la enfermedad de Chagas.

4. ADJUVANT EFFECT OF MPLA ON THE RECOMBINANT TSA-1ANTIGEN IN THE IMMUNOTHERAPY OF ACUTE *TRYPANOSOMA CRUZI* INFECTION IN BALB/C MICE

Juan José de la Cruz López, Leticia Chin Hau, Pedro Martínez Vega, Miguel Rosado Vallado, Eric Dumonteil

Laboratorio de Parasitología, Centro de Investigaciones Regionales "Dr Hideyo Noguchi", Universidad Autónoma de Yucatán, 97000 Mérida, Yucatán.

TSA-1antigen is under evaluation as candidate vaccine against Chagas disease, which previously demonstrated to reduced parasitemia, cardiac inflammation and increased host survival in mice and dogs, in both preventive and therapeutic models. Monophosphoryl lipid A (MPLA) has been used as adjuvants with other proteins for inducing Th1 immune response in mice. BALB/c mice infected with 500 trypomastigotes intraperitoneally were treated at 7 and 14 days postinfection with 20 μ g of recombinant TSA-1 added with 5, 10 or 15 μ g of monophosphoryl lipid A (MPLA), for further evaluation of immune response and control disease progression. In the evaluation of therapeutic vaccination to reduce pathology, the treatment with rTSA-1 and added with 10 μ g of MPLA showed the best results as significantly reduced parasitemia, cardiac parasite burden and density inflammatory cells. As well as induced higher antibody response inducing higher levels of antibodies anti-TSA-1 of the isotype IgG2a. Treatment with recombinant TSA-1antigen added with 10 μ g of MPLA induced the most complete control of disease consistent with a Th1-type immune response.

5. *TRYPANOSOMA CRUZI* INFECTION PREVALENCE AND BLOOD MEAL ANALYSIS IN VECTORS OF CHAGAS DISEASE IN SOUTHWEST TEXAS, 2013-2014

Rodion Gorchakov, MS¹; Edward J. Wozniak, DVM, PhD, MPH²; T. Patricia Fera, PhD³; Melissa N. Garcia, MPH¹; Peter J. Hotez, MD, PhD, FASTMH, FAAP¹; Kristy O. Murray, DVM, PhD¹

¹Department of Pediatrics, Section of Tropical Medicine, Baylor College of Medicine, Houston, TX; ²Zoonosis Control Unit, Texas Department of State Health Services, Uvalde, TX; ³Department of Biology, University of Texas Pan American, Edinburg, TX

Protozoan pathogen *Trypanosoma cruzi* (*T. cruzi*) is the etiologic agent of Chagas disease, which affects millions of people in Latin America and is an emerging public health threat in the United States. Transmission cycle of the parasite involves alternating infection of its vertebrate hosts and insect vectors. We identified the current vector infection burden and potential range of natural reservoirs of *T. cruzi* in 11 southwestern counties of Texas by analysis of insects of genus *Triatoma* collected during the period from June 2013 to January 2014. Out of 40 submitted specimens, the vast majority of the insects were *T. gerstaeckeri*, with only four samples of *T. sanguisuga*, two samples of *T. lecticularia*, and one sample of *T. rubida*. We found 73% of the insects PCR positive for *T. cruzi*. Blood meal analysis was performed on the infected triatomines. Blood sources were determined for all but one of the insects, and included 13 different species of mammals (mouse, woodrat, squirrel, porcupine, armadillo, cottontail, raccoon, fox, coyote, dog, pig, cow, human). Interestingly, 36% of the bugs were identified as having multiple blood sources. Since most of the insects were collected in or around residential houses, the most prevalent type of blood meal was human (50% of the insects). High infection rate of the triatomine vectors combined with high incidence of feeding on humans underscore the importance of Chagas disease surveillance in Texas. The results prompt urgent measures for screening of residents with a history of an insect bite for Chagas, vector control in and around homes, and increasing public awareness about the risk of Chagas.

6. IDENTIFICATION OF FEEDING HABITS OF *T. DIMIDIATA*, VECTOR OF CHAGAS DISEASE IN YUCATAN

Joel Moo, Eric Dumonteil, Etienne Waleckx

Universidad Autónoma de Yucatán, Centro de Investigaciones Regionales Dr. Hideyo Noguchi

Chagas disease is a zoonosis caused by the hemoflagellated protozoan *Trypanosoma cruzi*. This parasite is transmitted by hematophagous bugs called triatomines, which feed on a wide variety of domestic and wild vertebrates. In Yucatan, *T. dimidiata* is the only triatomine species reported. The accurate knowledge of *T. dimidiata* feeding patterns under natural conditions in Yucatan is critical information to develop adequate vector control strategies in the region. Here, we present, in a prospective way, a technique recently proposed we are planning to use for the identification of *T. dimidiata* blood meal sources in Yucatan. After purification of the total DNA present in the digestive tract of bugs collected in domestic and wild ecotopes in Yucatan, universal vertebrate primers will be used to amplify a 215 pb fragment of the 12S ribosomal RNA gene. The PCR products will then be cloned into the p-GEM-T Easy Vector System (Promega). DNA of the transformants will

then be reamplified before sequencing and inferring of the blood sources using BLAST. This work will help us to accurately describe the relationships between *T. dimidiata* and its different host in Yucatan.

7. NUTRITIONAL STATUS, FREQUENCY OF HUMAN BLOOD MEALS, AND TRYPANOSOMA CRUZI INFECTION OF TRIATOMA DIMIDIATA IN YUCATAN, MEXICO.

Silvia Pérez Carrillo, Eric Dumonteil, Etienne Waleckx

Universidad Autónoma de Yucatán, Centro de Investigaciones Regionales "Dr Hideyo Noguchi" Laboratorio de Parasitología.

In Yucatan, the causative agent of Chagas disease, *Trypanosoma cruzi*, is transmitted by the hematophagous bug *Triatoma dimidiata*. In order to evaluate human/vector/parasite contact, and clarify the parasite cycles of transmission in the region, we are currently assessing by PCR the presence of vertebrate and human blood meals, and the presence of the parasite in *T. dimidiata* specimens collected in sylvatic and domestic/peridomestic ecotopes in 3 rural communities of Yucatan. At this time, 137 bugs (63 males, 64 females, and six 5th instar nymphs) have been tested. *T. cruzi* infection was found in 29% of them; infection prevalence did not differ significantly between males (31%, 20/63) and females (29%, 19/64) (Fisher exact test, $p = 1$). The nymphs were not found infected. Vertebrate blood meals were detected in 50 of the bugs (60%). Of them, 5 (10%) were human blood meals. All human blood meals were detected in domestic/peridomestic bugs. Sixty percent of the bugs fed on human were infected with *T. cruzi*, revealing the risk of Chagas disease transmission to human. We are now working with cloning and sequencing to identify the other vertebrate blood meal sources, and to identify possible associations between *T. cruzi* infection and the different vertebrates. This will help us to accurately describe the parasite transmission cycles occurring in Yucatan.

8. PRE-EXPOSURE TO FECES OR SALIVA OF TRIATOMA DIMIDIATA DECREASES PARASITEMIA IN MICE CHALLENGED WITH TRYPANOSOMA CRUZI: DESCRIPTION OF INFLAMMATORY REACTION AT THE INOCULATION SITE

Victor Monteon¹, Enrique Quen-Ramírez¹, Oscar Hernández -Vázquez¹, Verónica Macedo-Reyes², Ruth Lopez³, Karla Acosta-Viana³, Pamela Pennington⁴, Angel Ramos-Ligonio⁵.

¹Centro de Investigaciones Biomédicas, Universidad Autónoma de Campeche, Campeche City, Mexico; ² Patología, Centro Oncológico de Campeche, Mexico; ³Centro Regional de Investigaciones Biomédicas, Universidad Autónoma de Yucatán, Méxic.; ⁴ Center for Health Studies, Universidad del Valle de Guatemala, Guatemala City, Guatemala; ⁵ LADISER Inmunología y Biología Molecular, Facultad de Ciencias Químicas, Orizaba, VER, Mexico.

Trypanosoma cruzi infection in natural conditions is transmitted to mammals when bug-derived feces that are contaminated with metacyclic trypomastigotes gain access through skin lesions, mucosa or bite wounds. In our work we mimic natural vector transmission in a murine model. We used saliva and feces from *Triatoma dimidiata* to inoculate experimental animals once or multiple times, before inoculation with feces contaminated with metacyclic trypomastigotes directly from *T. dimidiata*. The site of infection was analyzed for histological changes and parasitemia was followed by direct examination of blood in infected mice. We found that inoculation with saliva led to the recruitment of eosinophils and mononuclear cells at the inoculation site, whereas inoculation with feces led to the recruitment of neutrophils. Mice inoculated multiple times exhibited strong inflammatory reaction since the first hour. Mono- or multi-exposure to *T. dimidiata* fluids before inoculation with metacyclic trypomastigotes helped to control the level of parasitemia. In conclusion, previous contact with saliva or feces of *T. dimidiata* reduces parasitemia in *T. cruzi*-infected mice.

9. PROTEÍNAS INMUNOGÉNICAS PRESENTES EN LAS BACTERIAS CULTIVABLES EN HECES DE TRIATOMA DIMIDIATA

Iván Aarón May Gil, Víctor Monteon

Laboratorio de inmunoparasitología, Centro de investigaciones Biomédicas, Universidad Autónoma de Campeche

La enfermedad de Chagas es una enfermedad parasitaria transmitida por insectos triatominos de la familia *Rubidae*. El estudio de la microbiota de los triatominos por metagenoma o cultivo es de interés actual. En el presente trabajo se buscaron inmunógenos presentes en las bacterias de heces de *Triatoma dimidiata* que pudiesen servir como marcadores de exposición al triatomo. Se inmunizaron 10 ratones Balb/c con 5 microlitros de heces de *Triatoma dimidiata* cada semana durante 12 semanas, posteriormente se obtuvo el suero de cada ratón. El aislamiento de bacterias de heces de *Triatoma dimidiata* fue en agar sangre. Después de 48 horas se identificaron 7 morfologías coloniales, fueron aisladas y analizadas bioquímicamente -Vitek biomeriux. El extracto crudo de cada aislado obtenido por sonicación se analizó por SDS-PAGE y WESTERN-BLOT. De 7 colonias aisladas, 6 fueron identificadas como *Proteus*, *Pseudomonas* y *Citrobacter* y una no identificable. Los inmunógenos encontrados están entre 10 a 93 kDa en 4 de las colonias y 3 no presentaron reactividad. Las colonias 2 y 3 pertenecen al mismo género (*Proteus mirabilis*) pero sus inmunógenos tienen peso molecular distinto. Las bacterias aisladas de heces de *T. dimidiata* pertenecieron a *Proteus*, *Pseudomonas* y *Citrobacter*. Los inmunógenos reconocidos entre 10 a 93 kDa pudieran ser útiles como marcadores de exposición.

10. PROTEÍNAS INMUNOGÉNICAS DE REACCIÓN CRUZADA PRESENTES EN LA SALIVA DE TRIATOMA DIMIDIATA Y RHODNIUS PROLIXIUS.

Iván Aarón May Gil, Víctor Monteon, Karla Acosta Viana, Glendy Polanco

Laboratorio de inmunoparasitología, Centro de investigaciones Biomédicas, Universidad Autónoma de Campeche y Centro de Investigaciones Biomédicas "Hideyo Noguchi", Universidad Autónoma de Yucatán

Rhodnius prolixus y *Triatoma dimidiata* son dos de los principales vectores transmisores de la enfermedad de Chagas en América latina. Las moléculas en la saliva de estos insectos han sido un foco de interés para el área farmacéutica como para la epidemiológica. En el presente trabajo se determinaron las proteínas presentes en la saliva de *Rhodnius prolixus* que son reconocidas por el suero de ratones inmunizados con saliva de *Triatoma dimidiata*. Se inmunizaron 10 ratones Balb/c por exposición al piquete de *Triatoma dimidiata* cada 15 días por 3 meses, posteriormente se obtuvo el suero de los ratones.

La saliva de *Rhodnius prolixus* se obtuvo de 5 especímenes adultos mediante disección de la porción R3 de su probóscide. La composición proteica e inmunogénica de la saliva fue analizada mediante electroforesis bidimensional y WESTERN BLOT. De 43 proteínas en la saliva de *Rhodnius* Se encontraron 5 inmunógenos de reacción cruzada, estas proteínas están en un rango de peso molecular entre 12 a 60 kDa con puntos isoeléctricos que van de 4.8 a 7.1. 5 proteínas presentes en la saliva de *Rhodnius prolixus* son reconocidas por el suero de ratones expuestos al piquete de *Triatoma dimidiata*. Estas proteínas pudiesen servir como marcadores de exposición al piquete de estos 2 vectores

11. PURIFICATION AND CHARACTERIZATION OF A SOLUBLE LEISHMANIA DONOVANI NUCLEOSIDE HYDROLASE RECOMBINANT PROTEIN EXPRESSED IN PICHIA PASTORIS

Elissa Hudspeth[1][2]; Christopher A. Seid[1][2]; Molly Hammand[1][2]; Jeroen Pollet[1][2]; Wanderson C. Rezende[1][2]; Bin Zhan[1][2]; C. Patrick McAttee[1][2]; David A. Engler[3]; Rise K. Matsunami[3]; Peter J. Hotez[1][2][4]; and Maria Elena Bottazzi[1][2]

[1]National School of Tropical Medicine, Baylor College of Medicine; Houston, TX; [2]Sabin Vaccine Institute and Texas Children's Hospital Center for Vaccine Development, Houston, TX; [3]Proteomics Department, The Methodist Hospital Research Institute; Houston, TX; [4]James A. Baker III Institute for Public Policy, Rice University; Houston, TX

The intracellular parasite, *Leishmania*, creates an undue burden on rural populations throughout Africa, the Middle East, Asia, and South America through an infection causing cutaneous and visceral forms of Leishmaniasis. To date, no preventative vaccine has been developed. These parasites are unique from humans in that they must salvage and process purines from their host since they lack the ability for *de novo* purine synthesis. As part of this purine processing pathway a 36 kDa key nucleoside hydrolase (NH36) has been identified as a potential vaccine candidate antigen. Process development (fermentation and purification) and product characterization are essential steps to evaluate the feasibility of recombinant protein-based vaccine. Here we show the expression, purification, and characterization of NH36. Briefly, a 316 amino acid nucleoside hydrolase sequence from *Leishmania donovani* was cloned into a yeast expression system and evaluated as a potential vaccine candidate. Initial expression of a wild type clone in *Pichia pastoris* containing all six intact (putative) glycosylation sites resulted in a highly glycosylated protein with low expression yields. To improve expression and purification of the protein, four of the six putative glycosylation sites (N39, N77, N89, and N185) were mutated to glutamine residues (Q). The mutated construct was successfully expressed and purified through cation exchange chromatography (GE Capto SP Impress) followed by size exclusion chromatography (GE Superdex 200). Similar to the native nucleoside hydrolase, the protein was expressed as a tetramer with an approximate molecular weight of 137 kDa. Characterization studies of the recombinant protein performed by SDS-PAGE, Western blot, HPLC-SEC, deglycosylation assay, dynamic light scattering (DLS), and mass spectrometry of the purified recombinant protein will also be shown.

12. CUTANEOUS LEISHMANIASIS IN CUBAN IMMIGRANTS TO TEXAS WHO TRAVELED THROUGH THE DARIEN JUNGLE, PANAMA

Meagan A. Barry; Misha V. Koshelev; Kristy O. Murray; Laila Woc-Colburn
 Baylor College of Medicine, Houston, TX

Cutaneous leishmaniasis (CL) is rarely seen in the United States and the standard of therapy is lengthy and associated with frequent toxicities. New therapeutic options, such as liposomal amphotericin B are not yet widely utilized. This study is a case series of four Cuban immigrants to Texas who traveled by the same route, though all at different times, from Cuba to Quito, Ecuador, then by ground northward, on foot through the Darién jungle, Panama. These patients presented to the Tropical Medicine Clinic at the National School of Tropical Medicine, Baylor College of Medicine, Houston, TX with chronic ulcerative non-healing skin lesions and were diagnosed with *L. [V.] panamensis* by histologic analysis and species-specific polymerase chain reaction. All four patients were treated with liposomal amphotericin B at 3 mg/kg/day for 5 days, followed by 2 infusions at the same dose on days 14 and 21 to complete a total treatment of 21 mg/kg. During treatment, one patient experienced a mild self-resolving infusion-related reaction, with chills and a headache and three patients experienced an elevation of creatinine (2 times the normal value) that resolved within days. Two patients had good resolution of the lesions. One patient, who had the most extensive disease, with 13 lesions upon presentation, continued to have disease progression during liposomal amphotericin B treatment. Concern for treatment failure led to administration of the standard of therapy, sodium stibogluconate. This report highlights a previously underappreciated immigration route for Cubans through Central America, placing immigrants at risk for a number of emerging tropical diseases including CL. Physicians should be aware of this immigration route and include CL in the differential diagnosis for non-healing skin ulcers in this patient population. Liposomal amphotericin B can be a well-tolerated and efficacious treatment for CL caused by *L. [V.] panamensis*.

13. URINE-ENRICHED MEDIA FOR OPTIMIZING *L. (L.) MEXICANA* PROMASTIGOTES CULTURE PROCEDURE.

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Leishmaniasis is a disease caused by the flagellated protozoan of the *Leishmania* genus. This is transmitted by the bite of infected female sandflies. The diagnosis is through isolating of parasite in culture medium. Additionally, mass cultivation of *Leishmania* parasites is also needed in research laboratories for ELISA assays and experimental infections. The utilization of human urine as a constituent of *Leishmania* spp. culture medium, is an alternative to stimulate the growth of the promastigotes leading to a more rapid multiplication and a higher concentration of parasites at the stationary phase. Therefore, we evaluated the growth of *L. (L.) mexicana* in culture media enriched with fresh and frozen filtered human urine from a volunteer, and powder urea (0.5 mg/mL). Parasites growth was assessed qualitatively and quantitatively by microscopic observations and the number of parasites was counted every day using haemocytometer slide by ten days. Out of three media evaluated, the frozen and fresh urine have higher number of parasites with similar amounts in both cultures. Promastigotes were observed elongate and had rapid motility at the culture enriched with fresh urine compared with powder urea. However both fresh and frozen human urine made rosette forms that indicate appropriate culture conditions.

14. EVALUATION OF IMMUNE RESPONSE INDUCED BY INTRADERMAL AND INTRAMUSCULAR VACCINATION OF RECOMBINANT LEISHMANIA DONOVANI NUCLEOSIDE HYDROLASE (rNH36) USING GLUCOPYRANOSYL LIPID A/STABLE EMULSION (GLA-SE) AND E6020 AS ADJUVANTS IN BALB/C MICE.

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Leishmaniasis is the second largest parasitic killer in the world. (1) Vaccines are considered the best alternative for its treatment and prevention. rNH36 is a promising *Leishmania* vaccine candidate. (2-4) The inclusion of a TLR4 immune potentiator to a recombinant antigen vaccine enhances both the magnitude and the breadth of the engendered immune response. GLA-SE and E6020 are synthetic TLR4 agonists that demonstrated to enhance Th1 immune responses. (5,6) Furthermore the route of administration can influence the profile and magnitude of humoral and cellular immune responses because it determine the organs and cell populations involved in the resultant response.(7,8) Therefore the aim of this work was to evaluate and compare the immune response induced by intramuscular and intradermal administration of rNH36 with GLA-SE or E6020 in BALB/c mice. Female BALB/c mice (6-7 weeks old) were immunized at two weeks intervals with three doses of 10 µg of rNH36 and either 5 µg of GLA-SE or 5 µg of E6020 by intramuscular route into quadriceps muscle or intradermal route into ear dermis. Sera were collected two weeks after the last immunization and analyzed for antigen-specific IgG, IgG1, and IgG2a antibodies by ELISAs. Cells were isolated from spleen, 2x10⁶ splenocytes/well in triplicate were cultured for 22h with or without stimulation with 25 µg/mL rNH36 or with 20 µg/mL of concanavalin A. The frequency of CD3⁺/CD4⁺ and CD3⁺/CD8⁺ T cells producers of IFN-γ and IL-4 was determined by flow cytometry. Statistical analysis of antibody levels and the frequency of IFN-γ and IL-4 producing CD4⁺ and CD8⁺ T lymphocytes be performed comparing the experimental groups evaluated.

15. EVALUATION OF THE PROTECTION CONFERRED BY THE CANDIDATES TO PROHYLACTIC VACCINE NH36 EXPRESSED IN *ESCHERICHIA COLI* AND *PICHIA PASTORIS* AGAINST *LEISHMANIA (L.) MEXICANA* IN MICE

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Cutaneous leishmaniasis is one of the neglected diseases and is caused by the parasite *Leishmania (L.) mexicana*. The candidate to vaccine of recombinant protein NH36 produced in *E. coli* (1) has shown promising results. In an effort to optimize the Expression System has been changed, picking the yeast *P. pastoris* as the new E.S. and the route of administration(2). BALB/c mice were immunized using intradermal route with the following formulations: NH36^{E.coli}+GLA, NH36^{P.pastoris}+GLA, NH36^{E.coli}, NH36^{P.pastoris}, GLA and saline solution. Mice were infected with 1x10⁶ parasites and the inflammation was measured every week. We determined the levels of total IgG and isotypes against NH36 or soluble antigen. qPCR was used to estimate the parasite burden in the site of the infection. Mice immunized with NH36^{E.coli}+GLA had lower inflammation with significant difference (p<0.05) from week 9 post-infection and the same trend continued until the week 12 post-infection. The Group immunized with NH36^{E.coli}+GLA the parasite burden was lower than those mice immunized with NH36^{P.pastoris}+GLA. Aguilar-Be *et al.*, in 2005(3), tested the same vaccine with infected mice using 75% more of the NH36^{E.coli} protein but using the intramuscular route.

16. CYTOKINE MRNA EXPRESSION IN *PEROMYSCUS YUCATANICUS* (RODENTIA) INFECTED BY *LEISHMANIA (LEISHMANIA) MEXICANA*.

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Peromyscus yucatanicus the main reservoir of *Leishmania (Leishmania) mexicana* in the Yucatan peninsula of Mexico reproduces clinical and histological picture of LCL in humans as so subclinical infection. For these reasons, we have proposed its use as an experimental model to study LCL caused by *L. (L.) mexicana*. In this work, we analyzed cytokine mRNA expression in *P. yucatanicus* infected by *L. (L.) mexicana* to assess the immune response during pathogenic and nonpathogenic infections. Animals were infected with 2.5x10⁶ and 1x10² *L. (L.) mexicana* promastigotes. Cytokine expression was analyzed by real-time RT-PCR in skin at 4 and 12 weeks post-infection. At 4 wpi, high IFN-γ expression was observed in infected *P. yucatanicus*. No significant differences between *P. yucatanicus* inoculated with 1x10² and 2.5x10⁶ promastigotes were observed. At 12 wpi, IFN-γ and IL-10 expression was observed in *P. yucatanicus* with clinical and subclinical infection. The expression of TNF-α and IL-4 was significantly higher in clinical animals (2.5x10⁶) compared with subclinical ones (1x10²). High TGF-β expression was observed in *P. yucatanicus* with clinical signs when compared with uninfected animals. Results suggest a possible role of TNF-α, IL-4 and TGF-β in the immunopathogenesis of LCL in *P. yucatanicus* as in humans

17. FACTORES SOCIOCULTURALES ASOCIADOS A LA INFECCIÓN POR DENGUE EN NIÑOS DE EDUCACIÓN PRIMARIA EN YUCATÁN, MÉXICO

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En Yucatán se ha iniciado un estudio de cohorte prospectivo de infección por dengue en tres municipios (urbano, rural y costero). El estudio se basa en un enfoque interdisciplinario en el que la investigación cualitativa cumple el fin de mantenimiento del grupo de estudio a largo plazo. Se presentan los resultados preliminares en el área de estudio rural indígena con proceso medio de urbanización. Se realizó una entrevista en Profundidad, dirigida y estructurada, grabada y transcrita. Análisis de frecuencia discursiva. Aplicada al 10% de la muestra total de cohorte del área rural indígena (A 15 familias). Fueron Jefes de familias que conforman la cohorte de estudio cuyo miembro índice oscila entre 6 y 8 años de edad con Dispersión geográfica La preguntas fueron dirigidas al conocimiento de dengue, síntomas y duración de la enfermedad, transmisión y contagio, prevención y control. Resultados A pesar de la alta seropositividad la experiencia vivida es poco frecuente, por tanto, la percepción de riesgo es baja. La eliminación del vector es motivada por la molestia inmediata de picadura. Asociación de la picadura del mosquito como causa de la enfermedad, de la fiebre como principal síntoma y de la no acumulación de basura y agua como principal medida de prevención. La RS del dengue se forma a partir de la sintomatología que describen los programas de salud. El dengue se asocia a fiebre en el discurso, pero en la práctica la fiebre pocas veces se asocia a dengue. La fiebre pediátrica generalmente se asocia con algún tipo de infección del aparato respiratorio. La prevención se asocia directamente con la eliminación del vector. El uso del termómetro es poco frecuente en la población. Si la alta temperatura no imposibilita al niño no se considera que requiera atención médica. Los resultados obtenidos evidencian la importancia de los factores socioculturales asociados al dengue. La percepción social de las personas y familias en torno al binomio dengue-fiebre indican un punto clave para comprender cómo se construye culturalmente los discursos sobre enfermedad-atención-prevención. La ausente asociación causal entre fiebre y dengue en la vida diaria y su subsecuente automedicación, es un factor clave que puede estar contribuyendo al sub-registro de la enfermedad del dengue ya que se evidencia que el hecho de tener fiebre no indica que las familias consulten a los servicios de salud como primer recurso.

18. SEARCH DENGUE VIRUS IN AEDES (STEGOMYIA) AEGYPTI COLLECTED IN NON-RESIDENTIAL AREAS IN MERIDA, CITY, MEXICO.

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Currently in Yucatan State, dengue and chikungunya virus are cause of high morbidity in humans. Both viruses are transmitted by infected *Ae. aegypti*. Unfortunately, the control measures of the vectors are focused on households, but non-residential areas are a source of mosquitoes. The aim of the study is to determine the mosquito abundance (immature and adult) and search dengue virus in *Ae. aegypti*. Three times a week, we captured adult and collected immatures mosquito in churches (2) and schools (2) from July to October 2015. In total, at this moment has been collected 3,317 immatures in 24 water-filled containers. Most were *Ae. aegypti* immatures (2,669). Notably, we captured 8,276 adult of 12 species. Most were captured in churches (7,930), being more abundant *Culex quinquefasciatus* Say (4,407), *Ae. aegypti* (2,412), and *Ochlerotatus taeniorhynchus* Wiedemann (1,129). Other species recorded were *Culex nigripalpus* Theobald (151), *Culex interrogator* Dyar and Knab (83), *Culex salinarius* Coquillett (43), *Culex coronator* Dyar and Knab (19), *Aedes (Ochlerotatus) trivittatus* Coquillett (15), *Culex lactator* Dyar and Knab (14), and *Psorophora ferox* Humboldt (3). In female *Ae. aegypti*, 270 were unfed, 419 were fed and 140 were gravid. Currently, 125 female *Ae. aegypti* have been processed for identification of dengue virus and were negative. However, Churches and schools presented entomological risk.

19. INSECTICIDE RESISTANCE IN MOSQUITO *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) FROM YUCATÁN, MEXICO.

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Mosquitoes are major pest of importance in public health. The mosquito *Culex quinquefasciatus* is an important vector in temperate and tropical countries, highly anthropophilic and a major biting nuisance particularly in urban areas. The primary approach used to control transmission of these pathogens has mainly relied on the application of insecticides. However, mosquito-borne diseases are now resurgent, largely due to the difficulty in controlling vectors that have developed resistance to insecticides. The evolution of insecticide resistance by mosquitoes threatens human welfare as a result of their huge impact as vectors of human diseases. The loss of target site sensitivity to insecticides resulting from a substitution of leucine to phenylalanine, termed the *kdr* mutation, in the voltage-gated sodium channel of the insect nervous system is known to be important in insecticide resistance. The collecting of mosquito larvae was conducted in Hunucma, municipality located at west of Yucatán, during January through June, 2015. The larvae were carried to the laboratory where were reared. The genomic DNAs of 50 individual female adult mosquitoes were extracted by salt method. Presenting the 18% resistant individuals, 60% of individuals showed heterozygosity and 22% were susceptible. This result may be essential to establish control strategies integrated

20. *Aedes (Stegomyia) aegypti* IN CEMETERIES OF YUCATÁN, MÉXICO.

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Cemeteries are suitable habitats for mosquitoes due to the great availability of the different resources such as sugar substances, blood, shelter and water-filled containers. We examined the species composition and effect on *Ae. aegypti* immatures in containers (159) from July 2014 to September 2015 in eight cemeteries of Yucatán. In total, 24,948 specimens of nine species were recorded. The most commonly collected species were *Culex quinquefasciatus* Say (13,948), *Aedes (Stegomyia) aegypti* (6,482) and *Culex lactator* Dyar and Knab (3,573). Other species collected were *Culex interrogator* Dyar and Knab (498), *Culex nigripalpus* Theobald (357), *Culex coronator* Dyar and Knab (42), *Limatus durhamii* Theobald (22), *Aedes (Howardina) cozumelensis* Díaz Najera (15), and *Culex salinarius* Coquillett (11). There was statistical difference between the mean of *Ae. aegypti* immatures in the containers when it was alone and when cohabiting with other mosquitoes ($t = 2.051$, $df = 157$, $P = 0.042$). *Ae. aegypti* immatures alone in infested container was greater (4,711) compared to when co-habiting with other mosquitoes (1,771). In 68% (88/129) of the containers was found alone *Ae. aegypti*, while in 32% (41/129) was found cohabiting with other mosquitoes, most with *Cx. quinquefasciatus*. Cemeteries are an obligatory component of the rural and urban landscape and must be a priority site of control of annoying mosquitoes and disease vectors.

21. INSECTICIDE-TREATED HOUSE SCREENING REDUCE INFESTATIONS OF *Aedes aegypti* IN MERIDA, MEXICO

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We report the results of the evaluation of insecticide-treated house screening (ITS) to reduce infestations of *Aedes aegypti* in a cluster randomized controlled trial in Merida, a dengue-endemic Mexican city. A ITS-based intervention (Insecticide-treated nets Duranet Clarke® made of polyethylene with alphacypermethrin and permanently affixed to windows-doors with aluminum frames) was implemented and evaluated in 10 clusters (100 houses/cluster). Cross-sectional entomologic surveys quantified mosquito infestations at baseline (pre-intervention) and in four post-intervention samples surveys approximately at 6-monthly intervals corresponding to dry/rainy seasons sequentially over 2012-2014. Identical surveys were carried out in 10 control clusters that received no intervention. ITS resulted in immediate significant reductions of adult based-entomologic indicators, particularly for indoor-adult *Ae. aegypti* abundance (IRR≤41; $P < 0.05$) and on extended reductions of *Aedes* infested houses (OR≤0.62; $P < 0.05$) and their abundance (IRR≤0.57; $P < 0.05$) for 12-24 months. ITS prevents vectors from entering houses and is expected to protect for dengue and chikungunya both at personal and household-level. ITS was a popular intervention, and perceived efficacy was reinforced by a reduction in other domestic pests.

22. CONTROL OF *Aedes aegypti* BREEDING-SITES WITH THE PROGRAM RECICLA POR TU BIENESTAR IN MERIDA, MEXICO.

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We determined the most productive breeding-sites for *Ae. aegypti* in various neighborhoods of the Mexican city of Merida and evaluated the impact of a recycling governmental program "Recycle for your Commonweal" ("Recicla por tu bienestar" RxB) on the reduction of those breeding-sites and pupae production, and the perception of participants. Pre- and post-RxB entomological surveys were performed in a sample of houses (n=780) from 26 neighborhoods. A sample of houses (n=180) from six neighborhoods where RxB was not applied were considered as controls. A survey on the perception of participating people about dengue prevention and control and RxB was applied. Buckets/pots and "small diverse items" were the most important breeding-sites. RxB had a significant impact and reduced total containers (IRR = 0.74), positive containers (IRR = 0.33) and the risk of a house being positive for *Ae. aegypti* (OR = 0.41). All the interviewed participants referred RxB as necessary and most of them considered it useful. RxB should be considered as a good practice for the prevention and control of the vector of dengue and chikungunya

23. ECOLOGICAL NICHE MODEL FOR PREDICTING DISTRIBUTION OF *Aedes (Stegomyia) aegypti* IN YUCATÁN STATE, MÉXICO.

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The spread of dengue and chikungunya virus are determined by the distribution of *Aedes (Stegomyia) aegypti*. The objective of this study is to identify the geographic distribution of *Ae. aegypti* and measure the human population at risk of getting in contact with this mosquito species. We compiled distributional data for *Ae. aegypti* and other four species from several entomological studies in Yucatán between March 2010 and September 2014. Based on this data, we constructed ecological niche models to predict the spatial distribution of each species using the MaxEnt algorithm. Our models identified areas with suitable environments for *Ae. aegypti* in most of Yucatán. A similar percentage of urban (97.1%) and rural (96.5%) populations were contained in areas of highest suitability for *Ae. aegypti*, and no spatial pattern was found (Moran's $I = 0.33$, $P = 0.38$); however, we found association of abundance of immatures of this species with annual mean temperature ($r = 0.19$, $P < 0.000$) and annual precipitation ($r = 0.21$, $P < 0.000$). *Aedes cozumelensis* is also distributed in most areas of the Yucatán State; *Culex quinquefasciatus*, *Culex coronator* and *Culex thriambus* are restricted to the northwest. The information generated can help in decision-making to address the control measures in priority areas with presence of *Ae. aegypti*.

24. SEROLOGICAL EVIDENCE OF FLAVIVIRUS INFECTIONS IN PERIDOMESTIC RODENTS, IN MERIDA YUCATÁN, MEXICO.

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The aim of this study was to determine the presence of flavivirus infection in peridomestic rodents in Merida City, Mexico. Rodents were trapped inside 20 houses from October 2011 to September 2012 which were located within 100 meters of vacant lots that contained a substantial amount of domestic trash or vegetation. The capture was done with Sherman traps. Captured rodents were euthanized by CO₂ inhalation and sera was obtained by cardiac puncture and tested for flavivirus RNA by RT-PCR using a flavivirus-specific primers that target a ~250-nt region of NS5 gene and by PRNT using five mosquito-borne flaviviruses (Dengue virus-type 2, Dengue virus type-4, St. Louis encephalitis virus, West Nile virus and Yellow fever virus) and two rodent-associated flaviviruses that have no known arthropod vector (Apoi virus and Modoc virus). A total of 161 rodents were captured. Of these, 86 were *Mus musculus* and 75 were *Rattus rattus*. None of the rodents were positive by RT-PCR for flavivirus. Sixty-one (37.9%) rodents had neutralizing antibodies against at least one virus. Flavivirus seroprevalence rates were 64.0% for *R. rattus* and 15.1% for *M. musculus*. These data suggest that flavivirus commonly infect some species of rodents in Merida City.

25. NEW RECORDS OF ECTOPARASITES: *ECHINOLAELAPS ECHIDNINUS* AND *POLYPLAX SPINULOSA* IDENTIFIED ON RODENTS WITH SEROLOGICAL EVIDENCE OF FLAVIVIRUS INFECTIONS IN MÉRIDA, YUCATÁN, MÉXICO

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In México are scarce studies on the diversity of ectoparasites associated with rodents and limited regarding transmission of pathogens. In the present study, we report two new ectoparasites with the potential to be vectors of pathogens to humans. As part of a project to determine serological evidence of flavivirus infections in peridomestic rodents from Mérida City, México, we identified the ectoparasites in the rodents infected with virus (DENV-4), West Nile virus (WNV), Modoc virus (MODV), Apoi virus (APOIV) and yellow fever virus (YFV) in serum from rodents. We documented with photos two new records of ectoparasites. The spiny rat mite, *Echinolaelaps echidninus* (Berlese, 1887) and spined rat louse, *Polyplax spinulosa* (Burmeister 1839) were recorded for the first time in Yucatán State. The ectoparasites were identified mainly on black rat, *Rattus rattus*. Additionally, we report for the first time the flea *Ctenocephalides felis* (Bouché, 1835) recovered on *R. rattus*. *C. felis* is considered the main vector of filarial parasite *Acanthocheilonomum reconditum*, this parasite affect dogs and humans. In Yucatán, *C. felis* is vector of gram-negative bacteria known as *Rickettsia felis*. We discuss at work with particular reference to the potential for these arthropods to bite and transmit pathogens to humans.

26. EVIDENCE OF CHIKUNGUNYA VIRUS IN MOSQUITOS *Aedes (Stegomyia) Aegypti* FROM A RURAL COMMUNITY OF YUCATÁN, MÉXICO.

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Recently chikungunya virus (CHIKV) was introduced in Mexico and spread in various states of the Republic, including Yucatán, where there is currently an outbreak of the disease. To date, 816 CHIKV cases (official number by SSI) has been reported in the state of Yucatán. CHIKV is transmitted by infected *Ae. aegypti* to humans. **Methodology.** At this moment the capture was performed in 12 houses with suspected cases of chikungunya fever in the city of Mérida (n=11) and in the rural community of Ekmul, Tixkokob (n=1) during August to October 2015. In total, we collected 125 female *Ae. aegypti*, being 92 inside and 33 outside homes. They were grouped by pool, and then we perform RNA extraction with Trizol® technique. After the complimentary copy with Promega® Imprim II kit (cDNA) was performed. To determine virus infection technique we perform polymerase chain reaction point final. **Results.** We process a total of 25 pool and one pool (n=6 mosquitoes) was positive for infection CHIKV collected in the rural community by PCR. In addition we isolate CHIKV in cell line of *Aedes albopictus* (C6/36) and will start the process to sequencing. This is the first result that demonstrates the presence of CHIKV in *Ae. aegypti* collected in a home of a rural community of Yucatán.

27. CO-EXISTENCE OF *RICKETTSIA RICKETTSII* AND *RICKETTSIA FELIS* INFECTION IN *Rhipicephalus sanguineus* TICKS AND *Ctenocephalides felis* IN A MUNICIPALITY IN YUCATÁN, MÉXICO

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Rickettsiosis is an important febrile illness affecting Yucatán State population, usually confused as dengue fever. Since 1999, four *Rickettsia* species have been reported affecting humans in our state: *Rickettsia felis*, *Rickettsia akari*, *Rickettsia rickettsii*, and *Rickettsia typhi*. Search of *Rickettsia* species in ectoparasites from dogs in Yucatán was accomplished in houses of, 16 patients from Oxkutzcab municipality with previous Rickettsiosis diagnosis. Screen for *Rickettsia* species identification was accomplished by conventional PCR assay amplifying 3 different rickettsial DNA fragment genes. Obtained PCR products were sequenced. In our study, 6 houses had *Rickettsia* species presence, 2 houses had more than one *Rickettsia* species presence. A total of 28 *R. sanguineus* ticks were positive for *Rickettsia* identification, and 13 *C. felis* fleas respectively. DNA fragments obtained from tick samples had 99- 99.8% similarity to *R. rickettsii* str. Sheila Smith. DNA fragments obtained from flea samples had 99.4-99.7% similarity to *R. felis* URRWXCal2. More studies are required to determine the ecology, life cycle and geographical distribution of *Rickettsia* species in Yucatán State. Efforts should be focused in avoiding the misdiagnosis and rickettsiosis must be included in the differential diagnosis of the febrile illnesses.

28. TICKS FAUNA IDENTIFIED ON HUNTING DOGS (CANIS FAMILIARIS) IN A RURAL COMMUNITY OF YUCATÁN STATE, MÉXICO

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In Yucatán State the dogs have been commonly used for traditional hunting of wild vertebrates such as birds, mammals, and reptiles. The introduction of the dogs in the forest facilitates the transport of ectoparasites as ticks. This interaction host-ectoparasite promotes the infestation to the pets and farm animals, and usually humans. In the rural community of Chenché de las Torres, Temax, located in the northwestern of Yucatán State, was examined 18 hunting dogs with recent activity in November 2014 and ten dogs in May 2015. In total, 156 adult ticks of 12 species were recorded. The most commonly collected species were *Rhipicephalus sanguineus* Latreille (67), *Amblyomma americanum* Linnaeus (30), *Amblyomma inornatum* Banks (22), and *Ixodes scapularis* Say (12). Other species collected were *Amblyomma auricularium* Conil (7), *Amblyomma maculatum* Koch (2), *Dermacentor variabilis* Say (2), *Amblyomma longirostre* Koch (1), *Ixodes dentatus* Marx (1), *Ixodes baergi* Cooley & Kohls (1), *Haemaphysalis leporispalustris* Packard (1) and *Ornithodoros* sp. (1). Additionally were collected the dog flea *Ctenocephalides canis* Curtis (5) and the cat flea *Ctenocephalides felis felis* Bouché (4). Several of ticks identified in this study are potential vectors of pathogens, so its zoonotic role should be investigated locally.

29. TICKS (ACARI: IXODIDAE) FROM WILD MAMMALS IN FRAGMENTED ENVIRONMENTS IN THE SOUTH OF YUCATÁN PENINSULA, MÉXICO.

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In Latin America, sylvatic areas are used for ranching and food production, annually destroying large amounts of vegetation and leaving patches of forest for harvesting wood and hunting. Fragmentation increases the rate of contact between domestic and wild animals, exponentially augmenting pathogen exchange among species inhabiting such areas. From September 2012 to May 2013, ticks were collected from wild mammals ranging freely at Ejido Caoba and Ejido Laguna Om in the south of Quintana Roo State. The most abundant introduced vertebrate species at both sites were cattle (*Bos taurus indicus* L.), sheep (*Ovis orientalis* L.), and goats (*Capra aegagrus hircus* L.), for extensive farming. Thirty-five specimens of hard ticks (Ixodes) collected were *Amblyomma inornatum* Banks (18), followed by *Amblyomma ovale* Koch (7), *Amblyomma imitator* Kohls (3), *Haemaphysalis juxtakochi* Cooley (2), *Amblyomma auricularium* Conil (1), *Amblyomma maculatum* Koch (1), and *Amblyomma cajennense* Fabricius (1). The presence of ticks in fragmented landscapes, their introduction, and their role in transmission of pathogens remains unclear. The role of ticks as a bridge for pathogens between wild or domestic animals and humans, needs to be determined, as does the effect of ticks on health and productive status of wild and domesticated animals in fragmented environments.

30. DETECTION OF FILARIAL NEMATODES OF DOGS AND ARTHROPODS USING CONVENTIONAL PCR AND PAN-FILARIAL PRIMERS

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Filarial nematodes of dogs are pathogens affecting humans. In Mexico have been diagnosed *Dirofilaria immitis* and *Acanthocheilonomum reconditum* microscopically. However, worldwide exist at least other six filarial parasites can affect dogs and humans. *Pan-filarial* primers detect several species belonging to the *Onchocercidae* family. The aim of this study is determinate epidemiologic variables associated with infections in dogs, arthropods and humans from Tabasco, state, Mexico. November to May 2015, 337 blood samples of dogs have been collected and 124 processed by PCR. A total of 1,434 adult mosquitoes were captured and 13 different species of 7 genus have been identified: the both most abundant were *Culex quinquefasciatus* and *Mansonia perturbans* (40.7%). We recollected 172 adult ticks infesting sampled dogs, the most abundant were *Amblyomma maculatum* (90.7%) three species of fleas, the most abundant *Ctenocephalides felis* (40.6%) and two species of Lice the most abundant *Heterodoxus spiniger* (86.2%). At this moment the results shown a prevalence of 32.25%. The most samples amplified a product approximately to 542 pb compatible with *D. immitis* except eight samples amplifying products approximately to 578 pb compatible with *A. reconditum* and one sample amplifying a product approximately to 400 pb no compatible with previous reports. Data revealed a high prevalence of filarial infections in population of dogs sampled. However,

specific primers (CO₁) and sequencing technique should be used to determinate filarial species found.

31. GASTROINTESTINAL PARASITES FOUND IN COMMENSAL RODENTS FROM VILLAHERMOSA, TABASCO, MÉXICO.

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Rodents are usually found in urban environments and they are recognized vectors of parasites affecting public health. Human helminthiasis caused by parasites transmitting rats and mice occurs by the consumption of food or water contaminated with embryonated eggs, previously released from rodents. The aim of this study was to determine the presence of gastrointestinal parasites in commensal rodents from Villahermosa, Tabasco. Fifty rodents were captured alive from October to December 2014 randomly in the city using Sherman traps. Feces of rodents were collected and used to realize the Sheather's flotation technique and identify eggs of parasites. Three different species of rodents were captured, *R. rattus* (22%), *M. musculus* (32%) and *R. norvegicus* (46%). Capture sites were one glassmaking, one cyber coffee, two markets; the most were captured in houses. Fourteen different species of parasites were found in rodents: *Trichuris muris* (8%), *Eimeria* spp.(40%), *Nippostrongylus brasiliensis* (22%), *Nematospirides dubius* (34%), *Strongyloides ratti* (44%), *Syphacia muris* (6%), *Syphacia obvelata* (4%), *Heterakis spumosa* (6%), *Hymenolepis diminuta* (6%), *Hymenolepis nana* (16%), *Moniliformis moniliformis* (4%), *Trichosomoides crassicauda* (4%), *Mastophorus muris* (8%), *Toxocara* spp.(2%). Frequency of parasitosis according to the rodent specie was *R. norvegicus* (100%), *R. rattus* (82%) and *M. musculus* (44%). A high percentage of rodents (52%) presented mixed infections. A high frequency of gastrointestinal parasites were found in commensal rodents from Villahermosa, Tabasco.

32. PREVALENCIA Y FACTORES ASOCIADOS A PEDICULOSIS /ESCABIASIS EN ESCOLARES DE PRIMARIAS EN PETO, YUCATÁN.

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Las enfermedades por parásitos epidérmicos son enfermedades de cobertura mundial, relacionadas con pobreza y deficiencia en la higiene personal, generando estigma, actualmente se ha demostrado que se presenta en cualquier nivel social siendo más prevalente niños de 6 a 13 años, por tener mayor contacto durante el juego e interacción en el colegio. Se realizó un estudio observacional, descriptivo, transversal y prospectivo en la comunidad rural de Peto, Yucatán en escolares de escuelas primarias. Se determinó un tamaño de muestra de 67 niños (epíno v6) de 6 a 12 años, previo consentimiento informado y firmado por padres de familia se aplicó un cuestionario acerca de *Pediculosis Capitis* (PC) y Escabiosis y factores asociados, se realizó inspección de cabeza y cuero cabelludo con la técnica de wet combing o peine húmedo en búsqueda de liendres/piojos y un examen físico en la búsqueda de lesiones clínicas de dermatitis en zonas habituales de escabiosis. También se realizó un cuestionario a maestros y padres de familia acerca de factores asociados. 67 niños, 33 (49.25 %) varones y 34 (50.75 %) mujeres, con una edad media de 8 a 9 (intervalo 6-12 años).

De la inspección de cabello, cuero cabelludo y examen físico se encontró prevalencia de 37.3% (25/67) para PC. 12/25 (17.9 %) presentaron al mismo tiempo Escabiosis. Ser hombre o mujer no es factor de riesgo para presentar PC, tener de 10 a 12 años. Los hace más propensos a tener pediculosis con significancia estadística ($p = 0.028$). Las características del cabello, el tipo ondulado y el grosor delgado, tuvieron significancia estadística con una p de 0.037 y 0.024 respectivamente, los escolares con padres de 20 y 40 años de edad tienen mayor tendencia a presentar pediculosis con significancia estadística (padres de 20 a 40 años $p = 0.011$, madres de 20 a 40 años $p = 0.008$); en las madres la escolaridad primaria presentó significancia ($p = 0.009$) y ser empleadas u obreras en vez de trabajar en el hogar son factores para pediculosis con una significancia estadística de 0.057.

La prevalencia de Escabiosis fue 28.4% (19/67), 10 mujeres (29.4%) y 9 varones (27.2%), con p de 0.012 y 0.020 respectivamente, los escolares de 10 a 12 años presentaron una significancia estadística de 0.011, la edad de los padres fue factor que determinó la infestación con escabiosis (padres de 20 a 40 años $p = 0.002$, madres de 20 a 40 años $p = 0.004$). Con respecto a la escolaridad paterna: saben leer ($p = 0.027$), primaria ($p = 0.022$); tener 2 o más hermanos menores de 14 años en la misma casa está significativamente asociado $p = 0.004$. El compartir sábanas o ropa solo se encontró una asociación significativa en aquellos pacientes que comparten ropa con sus hermanos y la infestación de escabiosis ($p = 0.019$).

Los maestros definieron los meses entre Abril y Junio como los que presentan mayor incidencia de PC y Escabiosis, solo 18 % da aviso a las autoridades sanitaria, el 54.1 % deja que los alumnos continúen en clases mientras presentaron la infestación.

De los padres de familia encuestados el 40% tiene o ha tenido otro familiar con alguna de las ectoparasitosis y tan solo el 43 % acude al médico y de estos el 60 % recibe tratamiento ya sea oral, tópico u ambos y el 32.3 % aplicó el tratamiento a toda su familia.

33. VIGILANCIA TELEFÓNICA DE CASOS FEBRILES COMPATIBLES CON CASOS PROBABLES DE DENGUE EN UNA COHORTE DE NIÑOS ESCOLARES EN TRES COMUNIDADES URBANAS DE MÉRIDA, YUCATÁN, MÉXICO

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Describir la frecuencia de llamadas por cuadros febriles compatibles con casos de probable fiebre por dengue (FD) en una cohorte de escolares y sus familias, de acuerdo a sus (a) características sociodemográficas (b) geográficas 2. Caracterizar las fluctuaciones de llamadas por probable FD durante las semanas epidemiológicas 22-40 en el Sistema Nacional de Vigilancia Epidemiológica (SINAVE). Este estudio deriva del seguimiento telefónico de una cohorte prospectiva de dengue en niños en edad escolar (y sus familias), quienes fueron seleccionados mediante un muestreo aleatorio estratificado. Se analiza la frecuencia de llamadas entre las SE 22-40 y analizan en función de (a) características sociodemográficas de las familias (b) Su ubicación geográfica (c) La dinámica de transmisión de dengue en el entorno regional del SINAVE. Las llamadas por FD aumentaron en la SE 25, siendo las familias que residen en las zonas localizadas al sur de la ciudad de Mérida las que más llamaron, zona reportada como alta transmisión a dengue. Se observaron similitudes entre los patrones de frecuencia de llamadas por FD y los casos reportados en el SINAVE. La frecuencia de llamadas por FD en la cohorte muestra diferencias por estratos sociodemográficos y geográficos y guarda relación con el número de FD reportados en el SINAVE.

34. SLEEPING HABITS AFFECT ACCESS TO HOST BY CHAGAS DISEASE VECTOR TRIATOMA DIMIDIATA

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In the Yucatan peninsula (Mexico), the causative agent of Chagas disease *Trypanosoma cruzi* is transmitted by the bug *Triatoma dimidiata*. While *T. dimidiata* invades and colonizes houses in other regions, this species has an intrusive behavior in Yucatan, probably attracted by artificial light and potential hosts, but has limited ability to establish colonies. Bugs collected inside the homes also have a low nutritional status, suggesting that they cannot efficiently feed inside these houses. We hypothesized here that this low feeding status of *T. dimidiata* may be associated with the local practice in Mayan communities to sleep in hammocks instead of beds, as this sleeping habit could be an obstacle for triatomines to easily reach their host, particularly for nymphal instars which are unable to fly. To test this hypothesis, we used an experimental chamber of 100 cm x 50 cm x 50 cm in which we placed a miniature bed in one side and a miniature hammock on the other side. After placing a mouse enclosed in a small cage in the bed and another one in the hammock, *T. dimidiata* specimens were released in the chamber and their activity was video recorded (7 pm-7 am). Our results show that bugs were similarly attracted to both mice in the bed and in the hammock. However, they were able to reach the mouse located in the bed significantly more frequently than that located in the hammock. Adults reached the bed most frequently by walking, while they reached the hammock most frequently by flying. Interestingly, nymphs were also able, in few occasions, to reach the mouse in hammock by walking. Our conclusion is that sleeping in hammocks as in rural Yucatan makes the host less accessible to triatomines and may explain, at least in part, the low nutritional status and limited colonization of houses by *T. dimidiata* in the region.

35. PILOT INTERVENTION AGAINST TICK-BORNE DISEASES IN A MAYAN COMMUNITY

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Ticks are the second most important vectors not only because its worldwide distribution but also for the severe diseases that can transmit like rickettsiosis among others. People living in neglected communities, have a closer contact with ticks due to its economical activities like the agriculture, cattle-trade, hunting; or due to its poor living conditions. Therefore, intervention campaigns against vector borne diseases should consider the people knowledge and habits in the strategies aimed to reduce their exposition to those vectors. The objective of this work was to develop a pilot strategy that could be useful for the social intervention of Mayan communities from Yucatan, which considering their social and ecological characteristics are under constant risk of acquire tick borne diseases. This work has been developed in two phases. During the first phase we collected and analyzed the social determinants to design intervention campaigns against tick-borne diseases. The second phase consisted in the social intervention of children through talks, social cartography and ludic activities; and adults by workshops and community participation collects. Both interventions led to a strong empowerment of the knowledge by these people, which could have an impact in the reduction of tick-borne diseases.