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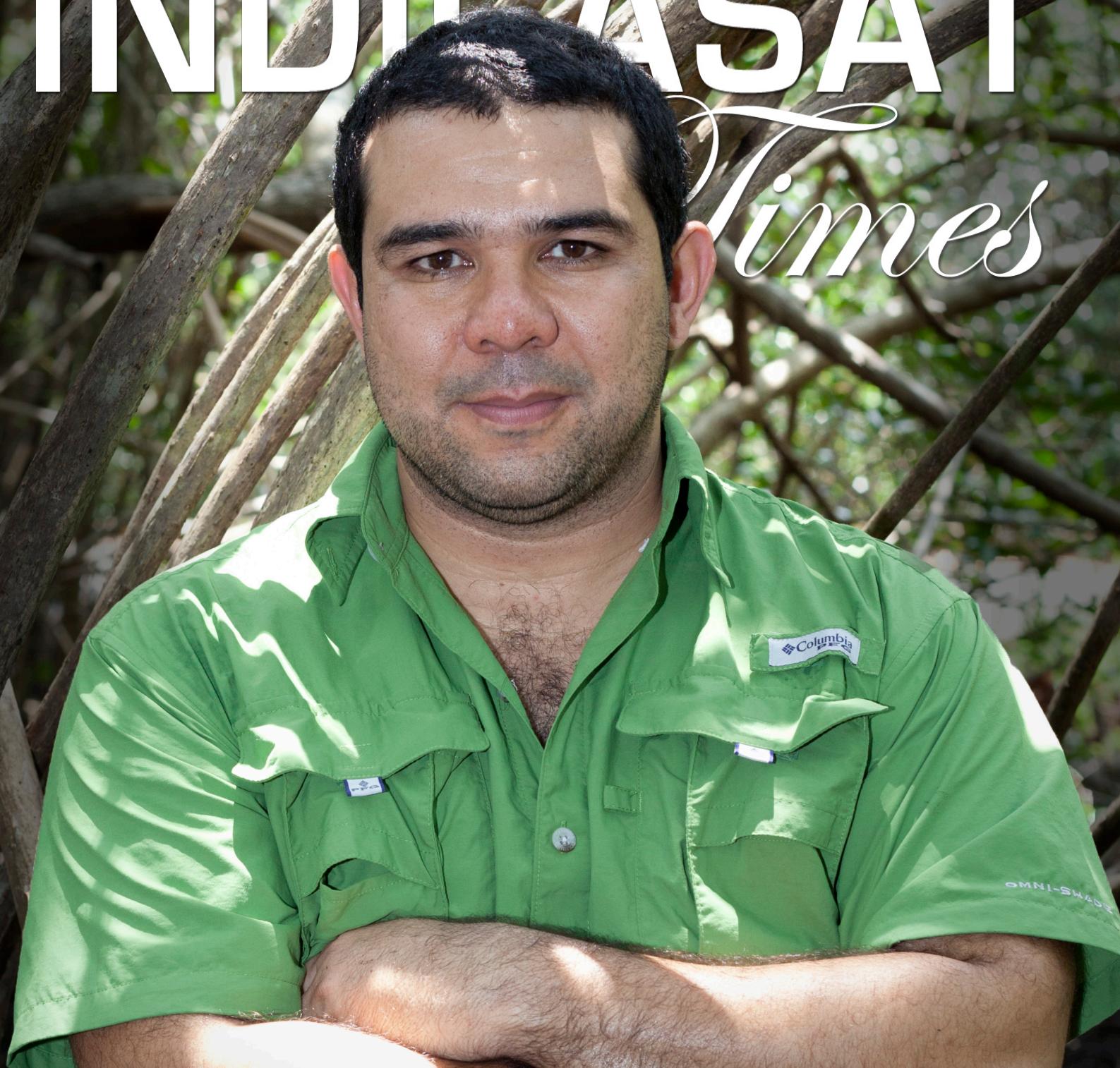




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THE INDICASAT TIMES | VOL. 3 (2) 2013 | ISSN 2222-7873



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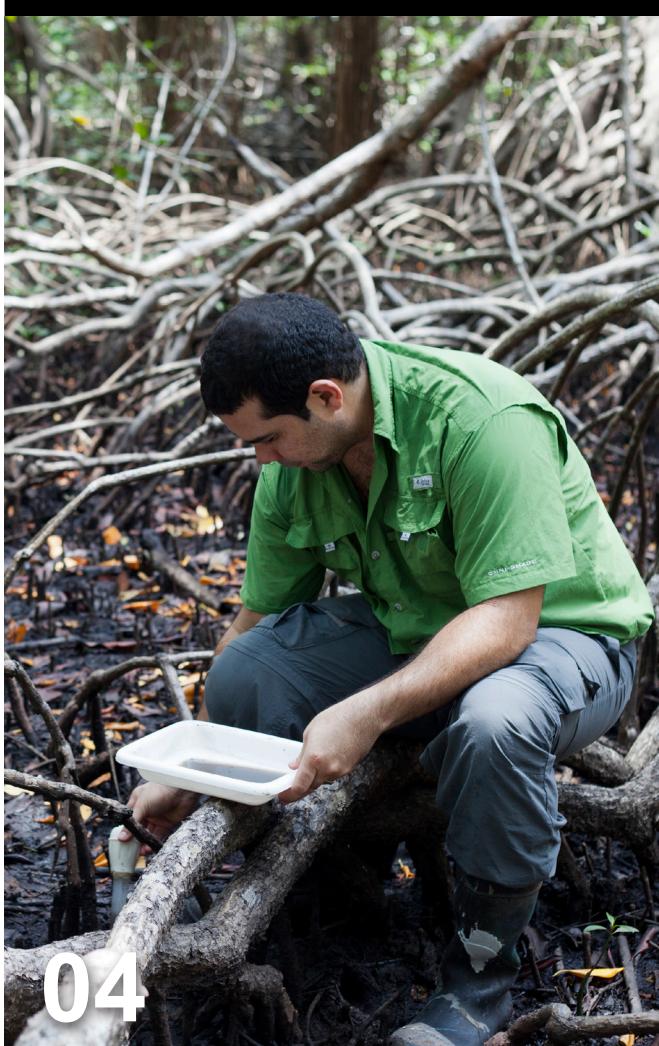
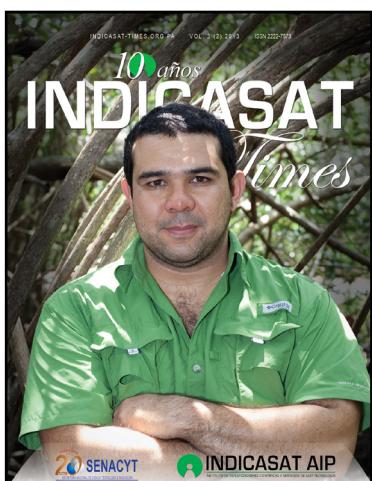
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PORTADA



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BIODIVERSI INSECTOS VECTORES Y ENFERMEDADES INFECCIOSAS

**“CONSERVAR LOS BOSQUES TROPICALES
PODRÍA AYUDAR A SALVAR VIDAS”**

JOSÉ R. LOAIZA PH. D.

**CENTRO DE DESCUBRIMIENTO DE DROGAS Y
BIODIVERSIDAD - INDICASAT AIP**

DAD



Photos by Rita Marissa Giovani





Panamá fungió como laboratorio natural para estudios de medicina tropical y enfermedades infecciosas, principalmente durante la construcción del Canal Interoceánico, a inicios del siglo pasado. Esto fue producto de numerosas batallas contra patógenos de la salud pública como el Plasmodio de la Malaria Humana y el virus de la Fiebre Amarilla. Un trágico devenir epidemiológico que resultó en elevada mortalidad y considerables pérdidas económicas. No obstante, estas epidemias contribuyeron a forjar la curiosidad científica que trascendió en el desarrollo de estrategias exitosas de mitigación y contención. La situación epidemiológica del país mejoró considerablemente entre 1940 y 1970, debido a la información científica generada por investigadores del Laboratorio Conmemorativo Gorgas, la creación del Servicio Nacional de Erradicación de la Malaria, y el uso de agentes químicos, por parte del Ministerio de Salud para eliminar a los insectos transmisores. Al presente, sin embargo, los esquemas de intervención contra enfermedades infecciosas permanecen inmutables y los esfuerzos en investigación sobre medicina tropical se han reducido significativamente. Todo esto aunado a las peculiaridades ambientales y demográficas del contexto actual, tales como, el acrecentado tránsito internacional, la pérdida de la biodiversidad y el cambio climático, han favorecido el resurgimiento de los males aludidos en otrora.

En el año 2004, Panamá reportó 5,095 casos de malaria, cifra no alcanzada en el país desde 1970. Mientras que en el 2011, 16 personas murieron por Dengue Hemorrágico, casi el doble de los decesos ocurridos en el lustro 2005-2010. A pesar del repunte epidémico de estas enfermedades, no existen hoy día hipótesis aceptadas sobre las causas de sus oscilaciones clínicas. Más alarmante aún, la existencia de cepas resistentes de *Plasmodium falciparum* (Samudio et al., 2005) y el descu-



brimiento de mosquitos vectores más eficientes, entre ellos, *Anopheles darlingi* en Darién y *Aedes albopictus* en Colón y Panamá (Loaiza et al., 2009) indican que existe un elevado riesgo de futuras epidemias en el país. Por otro lado, el panorama de las citadas Enfermedades Desatendidas o “Neglected Diseases” como el Mal de Chagas, las Arbovirosis y la Leishmaniasis no es más alentador. Aunque estas zoonosis no poseen la letalidad de otras infecciones tropicales, repuntan igualmente en la actualidad y causan notoria morbilidad en comunidades pobres de Panamá. Este es el caso particular de aquellas áreas intervenidas que presentan un elevado grado de deforestación. La conclusión que emerge de esta breve síntesis cronológica es la necesidad apremiante de fortalecer las investigaciones científicas sobre enfermedades infecciosas transmitidas por insectos vectores “Prevenir es más práctico que curar” (Prevention is always better than treatment) <http://www.cdc.gov>

Una de nuestras líneas de investigación, en el Centro de Descubrimiento de Drogas y Biodiversidad, se centra en el estudio de la relación entre los cambios del uso de la tierra, la pérdida de la diversidad biológica y la emergencia de enfermedades infecciosas. A la fecha hemos realizado trabajos en colaboración con el Instituto Smithsonian de Investigaciones Tropicales, el Departamento de Salud Pública del Estado de Nueva York (Wadsworth Center), el Walter Reed Biosystematics Unit y la Agencia de Protección Ambiental de los Estados Unidos de América. Específicamente, en el proyecto “Mosquito Species Diversity and Landscape Change” colectamos y analizamos datos ecológicos de insectos vectores para entender cómo las actividades antrópicas afectan su diversidad, abundancia y estructura comunitaria. Esta información es utilizada para predecir el riesgo de transmisión de patógenos en función del uso de las tierras

aledañas al bosque. Nuestros resultados indican que los cambios en la estructura comunitaria de los mosquitos no se deben a la distancia geográfica y/o variabilidad climática entre los sitios de muestreo, sino a los diferentes grados de perturbación humana que estos presentan. Conjuntamente, la riqueza de especies de mosquitos es menor en ambientes deforestados, pero la dominancia de algunas especies es mayor. Esto supondría un aumento en la transmisión de algunas enfermedades si sus vectores primarios son favorecidos demográficamente con los cambios en el uso de la tierra. Nuestros resultados preliminares apoyan esta hipótesis e indican que la tasa de infección de Arboviruses en mosquitos de áreas deforestadas es mayor que aquella de los colectados en sitios boscosos. Igualmente, la calidad y cantidad de hábitats larvales para mosquitos especialistas del bosque disminuye con la deforestación, la cual crea condiciones ecológicas distintas, resultando en un predominio de especies generalistas en áreas deforestadas. Estas especies generalistas son más eficientes en la transmisión de patógenos a humanos, ya que se adaptan a vivir en el peri-domicilio, se alimentan preferencialmente de nuestra sangre y sus poblaciones crecen exponencialmente (Vittor et al., 2006). Nuestros hallazgos resaltan el valor agregado de la biodiversidad de los bosques tropicales y su importancia para conservar el equilibrio ecológico y evitar el surgimiento de enfermedades infecciosas.

Nuestro interés por promover la investigación científica sobre Biodiversidad y Enfermedades Infecciosas en INDICASAT - AIP está encaminado a la formación de nuevos investigadores en áreas tales como ecología, biotecnología, entomología médica, genética poblacional, filogeografía y genómica. De igual manera, estamos promoviendo un mejor intercambio académico y tecnológico con instituciones nacionales e internacionales. En la actualidad el grupo que lidero está conformado por cinco estudiantes extranjeros (Estados Unidos de América, Salvador e Inglaterra) y cinco nacionales que cursan estudios en distintos

grados de escolaridad (Postdoctorado, Doctorado, Maestría y Licenciatura). En conjunto desarrollamos investigación científica básica y aplicada que se enmarca en las áreas prioritarias para la SENACYT y el Gobierno Nacional de "Biomedicina, Ciencias de la Salud" y "Biotecnología".

Los resultados de nuestros estudios permitirán optimizar la capacidad científico-técnica del país para mejorar la salud de los panameños y de los turistas que nos visitan. Sin embargo, para alcanzar estos objetivos será necesario acoger a la investigación científica como herramienta obligatoria para resolver los problemas de salud, al igual que aumentar los fondos para investigación.

Aspiramos a que INDICASAT AIP contribuya por medio de sus investigaciones a prevenir las enfermedades infecciosas transmitidas por insectos que resurgen en áreas ecológicamente alteradas del país.

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<http://www.cdc.gov>



Raster Image Correlation Spectroscopy in Live cells Expressing Endothelin ETA_A Receptor

Damaris De La Torre, Elizabeth A. Gordon, Michelle A. Digman, Milka Stakic, Hanns Häberlein, Enrico Gratton and Catherina Caballero-George.



Abstract

Fluorescence spectroscopy is the most common non-radioactive technique used to study GPCR interactions with their ligands. Raster image correlation spectroscopy (RICS) exploits spatio-temporal correlation functions rather than the simple temporal correlations of conventional fluorescence correlation spectroscopy. In this paper we describe the use of RICS and the number and brightness method to determine the diffusion of a construct of endothelin ETA_A receptor with EGFP and the aggregation state in the cytoplasm. Our construct seems to locate mainly in the cytoplasm where it undergoes diffusion and it appears to be monomeric. Although our construct could not fully represent the native protein, we believe that the methodology we describe in this paper could be used by anyone in this field.

Damaris De La Torre, Elizabeth A. Gordon, Michelle A. Digman, Milka Stakic, Hanns Häberlein, Enrico Gratton and Catherina Caballero-George. Current Trends in Biotechnology and Pharmacy, 6(5) 492-500 (2013).



Molecular phylogenetic relationships and phylogeography of *Anopheles triannulatus* complex (Diptera: Culicidae) support deep structure and complex patterns that are not congruent with formerly described species

Marta Moreno¹, Sara Bickersmith, Wesley Harlow, Teresa Fernandes Silva-do-Nascimento, Jose R. Loaiza, Jan E. Conn.

Abstract

The molecular phylogenetic relationships and population structure of the *Anopheles triannulatus* complex members: *Anopheles triannulatus* s.s., *Anopheles halophylus* and the putative species *Anopheles triannulatus* "C" were investigated. The mitochondrial COI gene, the nuclear white gene and rDNA ITS2 of samples that include the known geographic distribution of these taxa were analyzed. Phylogenetic analyses were performed with BI, MP and ML approaches. Each data set analyzed separately yielded a different topology, and none provided evidence for the separation of *An. halophylus* and *An. triannulatus* "C", consistent with the hypothesis that the two are undergoing incipient speciation. The phylogenetic analyses of the white gene found three main clades, whereas the statistical parsimony network detected only a single metapopulation of *Anopheles triannulatus* s.l. Seven COI lineages were detected by phylogenies and network analysis. In contrast, the network, but not the phylogenetic analyses, strongly supported three ITS2 groups. Combined data analyses provided the best resolution of the trees, with two major clades, Amazonian (clade I) and trans-Andean + Amazon Delta (clade II). Clade I consists of multiple subclades: *An. halophylus* + *An. triannulatus* "C"; trans-Andean Venezuela; central Amazonia + central Bolivia; Atlantic coastal forest; and Amazon delta. Clade II includes three subclades: Panama; cis-Andean Colombia; and cis-Venezuela. The Amazon delta specimens are in both clades, perhaps indicating local sympatry. Spatial and molecular variance analyses detected nine groups, some of which are concordant with these subclades. Most evidence indicates a trans-Andean origin for *An. triannulatus* s.l. The potential implications for malaria epidemiology remain to be investigated. **Marta Moreno¹, Sara Bickersmith, Wesley Harlow, Teresa Fernandes Silva-do-Nascimento, Jose R. Loaiza, Jan E. Conn. Parasites & Vectors, in press (2013).**



Coevolutionary patterns and diversification of ant-fungus associations in the asexual fungus-farming ant *Mycoceropurus smithii* in Panama

Katrin Kellner, Hermogenes Fernández-Marín, Heather D. Ishak, Ruchira Sen, Timothy A. Linksvayer and Ulrich G. Mueller.



Abstract

Partner fidelity through vertical symbiont transmission is thought to be the primary mechanism stabilizing cooperation in the mutualism between fungus-farming (attine) ants and their cultivated fungal symbionts. An alternate or additional mechanism could be adaptive partner or symbiont choice mediating horizontal cultivar transmission or de novo domestication of free-living fungi.

Using microsatellite-genotyping for the attine ant *Mycoceropurus smithii* and ITS rDNA-sequencing for fungal cultivars, we provide the first detailed population-genetic analysis of local ant-fungus associations to test for the relative importance of vertical versus horizontal transmission in a single attine species. *M. smithii* is the only known asexual attine ant, and it is furthermore exceptional because it cultivates a far greater cultivar diversity than any other attine ant. Cultivar switching could permit the ants to re-acquire cultivars after garden loss, to purge inferior cultivars that are locally mal-adapted or that accumulated deleterious mutations under long-term asexuality. Compared to other attine ants, symbiont choice and local adaptation of ant:fungus combinations may play a more important role than partner fidelity feedback in the co-evolutionary process of *M. smithii* and its fungal symbionts.

Katrin Kellner, Hermogenes Fernández-Marín, Heather D. Ishak, Ruchira Sen, Timothy A. Linksvayer and Ulrich G. Mueller. Journal of Evolutionary Biology, in press (2013).



Separation of Plasmodium falciparum late stage-infected erythrocytes by magnetic means

Lorena M. Coronado, Nicole M. Tayler, Ricardo Correa, Rita Marissa Giovani and Carmenza Spadafora.



Abstract

When *P. falciparum* infects the erythrocyte, the parasite degrades and feeds from haemoglobin (2, 3). However, the parasite must deal with a very toxic iron-containing haem moiety (4, 5). The parasite eludes its toxicity by transforming the haem into an inert crystal polymer called haemozoin (6, 7). This iron-containing molecule is stored in its food vacuole and the metal in it has an oxidative state which differs from the one in haem (8). The ferric state of iron in the haemozoin confers on it a paramagnetic property absent in uninfected erythrocytes. As the invading parasite reaches maturity, the content of haemozoin also increases (9), which bestows even more paramagnetism on the latest stages of *P. falciparum* inside the erythrocyte.

Based on this paramagnetic property, the latest stages of *P. falciparum* infected-red blood cells can be separated by passing the culture through a column containing magnetic beads. These beads become magnetic when the columns containing them are placed on a magnet holder. Infected RBCs, due to their paramagnetism, will then be trapped inside the column, while the flow-through will contain, for the most part, uninfected erythrocytes and those containing early stages of the parasite.

Here, we describe the methodology to enrich the population of late stage parasites with magnetic columns, which maintains good parasite viability (10). After performing this procedure, the unattached culture can be returned to an incubator to allow the remaining parasites to continue growing.

Lorena M. Coronado, Nicole M. Tayler, Ricardo Correa, Rita Marissa Giovani and Carmenza Spadafora.
Journal of Visualized Experiments, February 2013, e50342-page 1 of 4.

New partnerships

Two organizations have joined the Tuberculosis Biomarker program. The University Research Collaboration, LLC (URC) from Guatemala, Guatemala and the Instituto de Biomedicina, Caracas, Venezuela. The URC will provide training to health care workers in Colon for reinforcing the TB diagnosis and monitoring. The Instituto de Biomedicina, will collaborate in the evaluation of biomarkers for bovine tuberculosis and paratuberculosis diagnosis in Panamanian cattle industry.



Congratulations

Congratulations to Ciara Ordonez, Charybeth Chavarria and Wendy Franco from Dr. Amador Goodridge team for two abstracts accepted for poster presentations at the American Society for Microbiology (ASM) General Meeting in Denver, Colorado on May, 2013.

See abstracts below.

Basal levels of IgM anti-phospholipid antibodies in patients with pulmonary tuberculosis from a high-incidence setting in Panama before treatment initiation
Charybeth Chavarria^{1,2}, Wendy Franco^{1,2}, Ciara Ordoñez², Cheyenne Weeks², Anna Chavez³, Odemaris Luque³, Julio Jurado³, Ricardo Leonart², Lee Riley⁴, Amador Goodridge²

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para el Control de la Tuberculosis del Minsiterio de Salud – Colón; ⁴Division of Infectious Diseases and Vaccinology, School of Public Health, University of California at Berkeley

Background: Novel biomarkers for monitoring tuberculosis (TB) treatment are urgently needed to achieve disease control. We previously described the role of anti-phospholipid IgM antibody response in TB patients as a biomarker for monitoring TB therapy. We hypothesized that patients with pulmonary TB have higher levels of IgM anti-phospholipid antibodies before the initiation of directly observed therapy short-course (DOTS) than healthy, uninfected individuals. The present study determined the serum levels of IgM anti-phospholipid antibodies in patients with pulmonary TB at the time of diagnosis.

Methods: Between January 1st and December 31st 2012, 39 patients with pulmonary TB were recruited from Colon province in Panama. Demographic and clinical data were collected at the time of recruitment.

Serum samples were obtained from TB patients before undergoing DOTS and from 45 healthy donors.

Levels of IgM against cardiolipin (CL), phosphatidylethanolamine (PE) and sphingolipid (SL) were determined by ELISA.

Results: Our preliminary results show significantly higher levels of anti-CL, anti-PE and anti-SL IgM antibodies before DOTS compared to healthy donors ($p=0.0001$, 0.0001 and 0.0007 , respectively, Student's t-test). There were no significant differences in IgM antibody levels against the three phospholipids based on sex, age, nutrition status or diabetes diagnosis. The acid-fast smear test results and the presence of cavitary lesions on chest X-rays were not significantly associated with the levels of IgM anti-phospholipid antibodies.

Conclusion: These findings suggest that IgM anti-phospholipid antibodies are elevated in patients with pulmonary TB regardless of their clinical and demographic characteristics before DOTS therapy. Phospholipids induce the secretion of IgM anti-phospholipid antibodies by peritoneal cavity cells

Ciara Ordoñez¹, Cheyenne Weeks¹, Marla Ramos¹, Rene Rivera¹, Patricia Llanes¹, Nicole Baumgarth², Lee Riley³, Amador Goodridge¹

¹Centro de Biología Celular y Molecular de las Enfermedades at Instituto de Investigaciones Científicas Avanzadas – INDICASAT-AIP; ²Department Pathology, Microbiology and Immunology at Center for Comparative Medicine, University of California at Davis; ³Division of Infectious Diseases and Vaccinology, School of Public Health, University of California at Berkeley

Background: Novel biomarkers for monitoring tuberculosis (TB) treatment are urgently needed to achieve disease control. The innate immune response based on anti-phospholipid IgM antibodies has been proposed as a biomarker for successful TB therapy. We recently reported a decrease in anti-phospholipid IgM antibodies after two months of intense phase anti-TB therapy in patients with non-cavitory TB disease. Under normal physiological conditions in mice, bone marrow B-1 B cells sustain basal levels of these anti-phospholipid IgM antibodies; however, bacterial infection increases the serum levels of these IgM

antibodies. Infection with *Mycobacterium bovis* Bacille Calmette–Guérin (BCG) in mice has been shown to increase total B-1 B cells in the lungs, mainly produced by an influx of B-1a B cell subset from peritoneal cavity. We hypothesize that phospholipids released during TB lung pathology activate the secretion of anti-phospholipid IgM antibodies from B-1 B cells.

Methodology: The present study determined the levels of anti-phospholipid IgM antibody secreted in vitro from total peritoneal and spleen cavity cells after stimulation with phospholipids. Peritoneal and spleen cells were obtained from un-infected, eight-week-old, C57BL/6 mice and stimulated with cardiolipin (CL), phosphatidylcholine (PTC), phosphatidylinositol (PI) and sphingolipid (SL). The secretion of IgM antibodies against CL, PTC, PI and SL by peritoneal and spleen cells was measured by ELISA. The percentage of peritoneal B-1 B cells was determined by flow cytometry.

Results: Our preliminary results show 30% of peritoneal cells as B-1a B cells (CD5+CD43+). The levels of IgM anti-phospholipid antibodies increased in the peritoneal cell culture but not in the spleen cell culture after seven days of incubation with phospholipid stimuli. PTC and PI significantly induced the secretion of IgM anti-PTC by peritoneal cells. PTC also significantly induced the secretion of IgM anti-CL after seven days of culture.

Conclusion: These findings suggest that phospholipids are able to activate the secretion of IgM anti-phospholipid antibodies by peritoneal cells but not by splenocytes.



Miss. Erika Guerrero has won IBRO-SFN award of \$2000, for her research on Synuclein-DNA complex role in Parkinson Disease. She presents her work in SFN, 2013 in San Diego, USA. She is the first Panamanian to win this award.

Congratulations

Congratulations to Dr. Patricia Llanes for winning the Global Health Travel Award to attend the Keystone Symposium “The innate immune response in the pathogenesis of infectious diseases”, to be held at the Federal University of Ouro Preto, Brazil.



Dr. Jagannatha Rao is elected as Editorial Board Member of Asian Journal of Neuroscience 2013 to 2025

Dr. Ruben Berrocal and Dr. Rao research paper on “Studies on the mechanism of the DNA nicking property of Amyloid- β 40: Implications in Alzheimer’s disease.” as a significant contributor to the study of Alzheimer’s disease (AD). It is of special interest to the progress in this Psychology field. We would like to list your publication on our next edition of the Psychology Progress series. Psychology Progress alerts the scientific community to breaking journal articles considered to represent the best in Psychology research. James L. Robertson, VP Academic Affairs, Psychology Progress 1390 Prince of Wales Drive Suite 202, Ottawa, ON K2C 3N6

Why is the FDA Worried About Your Daily Caffeine?

By Meghan Foley | More Articles

May 04, 2013

Caffeine was once a stimulant found only in simple cups of coffee or black tea, and once the U.S. Food and Drug Administration approved use for a single food in the 1950s, cola was added to that list.

Now, sixty-years later, a bizarre range of caffeinated products occupy grocery-store shelves — from Wrigley's Alert Energy Gum to Wired Waffles

to an espresso bean candy called Crackheads — and the FDA has become a little worried about the effects of all this caffeine on the health of America.

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The agency is considering placing limits on just how much caffeine manufacturers can stick in their food products, restricting what foods can be infused with the substance, and requiring mandatory labels warning that these highly caffeinated products are not intended for children.



Already for NCAA athletes, too much caffeine is considered a performance-enhancing drug, and science has detailed quite a list of the disturbing side effects of the drug: restlessness, mood swings, and dehydration. It can even contribute to heart problems such as mild arrhythmia...

"What we're finding disturbing is this progression of caffeination of the food environment," Michael Taylor, the FDA's deputy commissioner for food, told The Wall Street Journal. "There may be a need to impose some limits on the amount of caffeine in

certain products.” Caffeine infusion may not be appropriate at all for some products, he added. NEW! Discover a new stock idea each week for less than the cost of 1 trade. CLICK HERE for your Weekly Stock Cheat Sheets NOW!

The primary concern is that the culinary landscape of America has changed and caffeine has cropped up in foods that federal regulators would never have imagined in the 1950s. “The only time that FDA explicitly approved the added use of caffeine in a food was for cola and that was in the 1950s,” said Taylor in a statement on April 29.

“Today, the environment has changed. Children and adolescents may be exposed to caffeine beyond those foods in which caffeine is naturally found and beyond anything FDA envisioned when it made the determination regarding caffeine in cola.”

Because of these new sources of caffeine, the FDA has determined it must examine the potential impact that the stimulant may have on health, especially with regard to vulnerable populations like children...

The FDA’s investigation has taken a few tentative steps forward; the agency has contacted several companies — including Mars, Kraft (NASDAQ:KRFT), and PepsiCo (NYSE:PEP) — about its caffeine concerns, which include energy drinks. “We’ll gather as much information as we can on the products that are out there and look at what should be the limits and what are the options for us to put some boundaries on the proliferation of caffeine in foods,” the deputy commissioner explained.

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It is not any one particular item on the market that has drawn the attention of the FDA, but



rather the “proliferation” of foods with added caffeine. “Any individual product is not a public health problem, but how do we look at the [increased] cumulative intake, and the norms that should govern this?” said Taylor. Currently, there are no requirements for labeling or limiting the addition of caffeine to foods, except for soda where the stimulant is restricted to 200 parts per million. One possible option under consideration by the FDA is to add a cautionary warning on products about the appropriate use of the product. The only problem is that in caffeine-crazed America, it is unclear whether such a label will deter or inadvertently appeal to consumers.



Prevalence of infection with bovine TB

Herd of *Bos Taurus* subsp. Santa Gertrudis
302 animals dispersed > 1 year

Y-interferon	18 positives	7 yielded <i>M. bovis</i>
PPD	0 positives	

- Prevalence of Bovine TB with PPD bovine 0%
- Prevalence of Bovine TB with Y-interferon test 2.5%
(based on culture positive animals)



CONFERENCIAS



Foto Rita Marissa Giovani

SENACYT-Panama-USA Dimensions of Biodiversity Workshop, 9th and April 10th, 2013





REUNIÓN PARA REVISIÓN DEL SUBSIDIO OTORGADO POR MELO ACERCA DEL ENVEJECIMIENTO CEREBRAL



VISITAS RECIENTES



Visita de estudiantes de la Facultad de Medicina de la Universidad de Panamá, de la licenciatura en Tecnología Médica.



Visita de la Ing. Miriam Rubio, Secretaria de SENACYT , Guatemala, junto al Dr. Ricardo Leonart, INDICASAT AIP y la Licda. Silvia Paiz, Directora de Relaciones Internacionales.



Visita de la Sra. María Luisa Picard-Ami y la Dra. Kathleen Curtis, de la Universidad de Texas en El Paso, Facultad de Ciencias de la Salud, junto al Dr. José Loaiza, el Dr. Jagannatha Rao y la Licda. Yeni Morales.



Visita de Consultores de Earth Train junto a Equipo de INDICASAT AIP.



PANAMA AS AN INTERNATIONAL SCIENCE HUB

