BiOTicks 2019

The Challenge of Tick Control

From December 1st to 4th / Del 1 al 4 de Diciembre

Organized by /Organizado por el Centro de Ingeniería Genética y Biotecnología, Habana, Cuba

Venue /Sede: Hotel Paradisus Varadero, Varadero, Matanzas, Cuba

http://www.paradisusvaraderoresort.com/


Organizing Committee/Comité Organizador

Presidents/Presidentes: Mario Pablo Estrada García/ Eulogio Pimentel Vázquez

Scientific program/Programa científico: Alina Rodríguez Mallon

General program/Programa general: Cigry Pérez, Tatiana Vázquez, Lincidio Pérez.

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Commercial exhibition/Feria comercial: Hector Machado

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General Program/ Programa General

Sunday December 1st	**Monday December 2nd**	**Tuesday December 3rd**	**Wednesday December 4th**
8:30-11:30 Transfer to hotel	8:30-10:30 Oral Session	8:30-10:30 Oral Session	8:30-9:30 Closure Ceremony - Check out
	10:30-11:00 Coffee Break – Poster Discussion	10:30-11:00 Coffee Break– Poster Discussion	10:00 Touristic Excursion
11:30-15:00 Check in - Registration	11:00-13:30 Oral Session	11:00-13:30 Oral Session	
Lunch	13:30-15:00 Lunch	13:30-15:00 Lunch	Farewell Lunch
17:00-19:00 Inaugural Ceremony	15:00-17:30 Oral Session	15:00-17:00 Oral Session 17:00-17:30 Poster Discussion	Touristic Excursion and transfer to Havana city
19:00-21:00 Welcome Dinner		20:00 Cultural Activity with traditional Cuban music	
21:00-23:00 Welcome party			

Sunday December 1st /Domingo 1 de Diciembre

8:30-11:30 Bus transfer Havana-Paradisus Varadero Hotel / Traslado Habana-Hotel Paradisus Varadero

11:30-17:00 Registration to the Congress/ Acreditación al Congreso

17:00-19:00 Inaugural Ceremony / Acto Inaugural
Room Cárdenas I / Sala Cárdenas I

17:00-17:20 Welcome to BioTicks 2019 and General information about the Congress
Dr. Alina Rodríguez Mallon
Center for Genetic Engineering and Biotechnology, Havana, Cuba

17:20-18:20 The Cuban Biotechnology: A Vaccine Platform in frame with One Health Program
Dr. Mario Pablo Estrada
Center for Genetic Engineering and Biotechnology, Havana, Cuba

19:00 -21:00 Welcome dinner/ Cena de Bienvenida
Bana Restaurant/ Restaurante Bana

21:00 -23:00 Welcome party / Fiesta de Bienvenida
Swimming pool area /Área de la piscina

Monday, December 2nd /Lunes, 2 de Diciembre

Room Cárdenas I / Sala Cárdenas I

Tick Control

Chairperson: Dr. Johann Schröder

8:30-9:00 Controlling ticks on cattle under different epidemiological conditions.
Dr. Johann Schröder
Program Manager, Animal Health, Welfare and Biosecurity, Meat & Livestock Australia

9:00-9:30 From tick biology to the rational design of novel ‘anti-tick‘ strategies
Dr. Petr Kopáček
Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

9:30-10:00 Skin microbiota in outcomes of tick infestations: implications for development of immunobiological, semiochemical and probiotic interventions to reduce tick loads in cattle.
Dr. Isabel Kinney Ferreira de Miranda Santos
Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo

10:00-10:30 Development of a tick vaccine with improved efficacy against *Rhipicephalus microplus* ticks.
Dr. Theo Schetters
Clinglobal, Mauritius and Department of Veterinary Tropical Diseases, University of Pretoria, South Africa

10:30-11:00 Coffee Break –Poster Discussion/ Receso-Discusión de Carteles

11:00-11:30 Tick immunological control as a feasible alternative in field conditions.
Dr. Alina Rodríguez Mallon
Center for Genetic Engineering and Biotechnology, Havana, Cuba

11:30-12:00 Vaccines candidates against ticks based on the conjugation of P0 peptide to three carrier proteins
Dr. Luis Javier González
Center for Genetic Engineering and Biotechnology, Havana, Cuba

12:00-12:30 Understanding tick sialome towards vector and pathogen control
Dr. Ana Domingos
Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal

12:30-13:00 *In silico* analysis of proteins from salivary glands of *Amblyomma cajennense* for the identification of conserved antigens.
Dr. Octavio Merino
Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, México

13:00-13:30 From transcriptome analysis of *Ixodes ricinus* synganglion to functional characterization of neuroreceptors
Dr. Olivier Plantard
BIOEPAR, INRA, Oniris, Nantes, France

13:30-15:00 Lunch/Almuerzo

Room Cárdenas I / Sala Cárdenas I

Ectoparasite Control

Chairperson: Dr. Gervasio Bechara

15:00-15:30 Control of *Ichthyophthirius multifiliis* (Ich) by mucosal immunoglobulins: Novel findings and implications for the design of effective vaccines against Ich.
Dr. J. Oriol Sunyer
School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA.

15.30-16:00 Vaccines against sea lice, a promising alternative but still challenging
Dr. Yamila Carpio González
Center for Genetic Engineering and Biotechnology, Havana, Cuba

16:00-16:30 Manejo integral de los tratamientos garrapaticidas
Dr. Eduardo Ramírez España
Gerente Nacional Técnico Bovinos de pastoreo, Bayer de México

16:30-17:00 Experiencias en el uso de una vacuna recombinante de Bm86 contra la garrapata *Rhipicephalus microplus* en México.
Dr. Martin Ortiz
LAPISA, Michoacán, México.

17:00-17:30 CIGB collaborations with CAMEVET: Gavac, the first subunit immunogen registered in a document with a format approved for the Americas.
Dr. Jesús Mena
Center for Genetic Engineering and Biotechnology, Havana, Cuba

Tuesday, December 3rd /Martes 3 de Diciembre

Room Cárdenas I / Sala Cárdenas I

Tick-borne diseases

Chairperson: Dr. Sara Moutailler

8:30-9:00 High-throughput nanotechnologies for tick-borne pathogens detection
Dr. Sara Moutailler
Animal Health Laboratory, UMR BIPAR, ANSES, INRA, ENVA, Paris University, France

9:00-9:30 Resistance of tick microbiota to biological disturbance
Dr. Alejandro Cabezas Cruz
Department of Animal Health, National Institute for Agricultural Research, INRA, France

9:30-10:00 An integrated approach for the molecular identification of ticks and detection of tick-borne diseases
Dr. Luis M. Hernández-Triana
Animal and Plant Health Agency, Virology Department, Woodham Lane, Addlestone, Surrey, United Kingdom

10:00-10:30 Current Fever Tick Research in Texas
Dr. Dee Ellis, DVM MPA
Institute for Infectious Animal Diseases (IIAD), Texas A&M University System, USA

10:30-11:00 Coffee Break –Poster Discussion/ Receso-Discusión de Carteles

11:00-11:30 Severe fever with thrombocytopenia syndrome virus from ticks and animals
Dr. Joon-Seok Chae
Laboratory of Veterinary Internal Medicine, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Republic of Korea.

11:30-12:00 Genetic diversity of *Anaplasma marginale* in cattle from Itú, state of São Paulo, southeastern Brazil
Dr. Rosangela Zacarias Machado
Facultad de Ciencias Agrarias y Veterinarias. Universidad Estadual Paulista (FCAV-UNESP), Sao Paulo, Brasil

12:00-12:30 Situation of bovine hemoparasitosis transmitted by *Rhipicephalus microplus* in Cuba
DMV. Rafmary Rodríguez Fernández, MSc.
National Laboratory of Parasitology, Cuba

12:30-13:00 Dynamics of genomic variations in *Leishmania panamensis* during the generation of resistance to trivalent antimonial
Dr. Ricardo L. Lleonart Cruz
Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), Panamá

13:00-13:30 Trade-off between allergy and protection to tick-borne diseases
Dr. Alejandro Cabezas Cruz
Department of Animal Health, National Institute for Agricultural Research, INRA, France

13:30-15:00 Lunch/Almuerzo

Room Cárdenas I / Sala Cárdenas I

Tick taxonomy and epidemiology

Chairperson: Dr. Filipe Dantas Torres

15:00-15:30 The *Rhipicephalus sanguineus* complex: What's next?
Dr. Filipe Dantas Torres
Instituto Aggeu Magalhães, Fundação Oswaldo Cruz (Fiocruz), Recife, Pernambuco, Brazil

15:30-16:00 *Rhipicephalus sanguineus* tropical and temperate lineage: a proteomic overview
Dr. Gustavo Serón
Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal

16:00-16:30 Current taxonomic status of the main Ixodid ticks with veterinary and public health interest in Cuba.
DMV. Pedro Encinosa Guzmán, MSc.
Center for Genetic Engineering and Biotechnology, Havana, Cuba

16:30-17:00 The potential role of migratory birds in the spread of ticks and tick-borne pathogens in Baltic region
Dr. Algimantas Paulauskas
Department of Biology, Vytautas Magnus University, Kaunas, Lithuania

17:00-17:30 Poster Discussion / Discusión de Carteles

Wednesday, December 4th /Miércoles 4 de Diciembre

Room Caonao I / Sala Caonao I

8:30-9:30 Closure Ceremony/ Acto de Clausura
Dr. Mario Pablo Estrada
Center for Genetic Engineering and Biotechnology, Havana, Cuba

10:00 Bus departure from the hotel Paradisus Varadero to Bellamar Caves Excursion and city tour around Matanzas / Salida de ómnibus desde el hotel Paradisus Varadero hacia Excursión a la Cuevas de Bellamar y paseo por la ciudad de Matanzas

16:30-19:30 Transfer Bus Matanzas-Havana / Traslado Matanzas-Habana

CONFERENCES/CONFERENCIAS ABSTRACTS/RESÚMENES

Conference abstracts are organized by the presentation order in oral sessions

Los resúmenes de las conferencias están organizados por el orden de presentación en las sesiones orales

The Cuban Biotechnology: A Vaccine Platform in frame with One Health Program

Mario Pablo Estrada, Alina Rodríguez-Mallon, Marisela Suárez, Yamila Carpio, Lídice Mendez

E-mail: mario.pablo@cigb.edu.cu
Center for Genetic Engineering and Biotechnology. P.O. Box 6162, Habana 10600, Cuba.

Abstract/Resumen

The practice of vaccination for the prevention of animal diseases has been used for centuries and has proven to be a powerful tool for the alleviation of animal suffering as well as the economic well-being of producers of animal products. The associated evolution to new technologies in the field of molecular biology and immunology has furthermore had a large impact on the development of new vaccine strategies and the quality of the products that are produced. The Center for Genetic Engineering and Biotechnology of Havana has created a platform to produce different and complex antigens for veterinary vaccines in order to satisfy the necessity of animal health in Cuba. Antigens produced in *E. coli* (bacteria), *P. pastoris* (yeast) and HEK293 or CHO (mammalian cells) have been probed as veterinary vaccine immunogens. The impact in the control of cattle ticks for more than 15 years using an immunogen produced in yeast, as vaccine, is one strong example of the possibilities of this technology. Other three vaccine candidates to control ectoparasites in salmon, classical swine fever virus and rabbit hemorrhagic disease virus have been produced, in three different expression systems, which have demonstrated efficacy and potential success in their applications. These results using our subunit vaccines have demonstrated advantages compared to live attenuated or inactivated virus vaccines, because apart from its ability to induce strong humoral and cell-mediated immune response, some of them have been developed with a DIVA diagnostic system. This is the case of our vaccine against classical swine fever virus that could be used both in the control stage and in the eradication of the disease in Cuba. Furthermore they have shown an excellent safety profile and a robust immune response against pathogens.

Controlling ticks on cattle under different epidemiological conditions

Johann Schröder

Email: jschroder@mla.com.au
Program Manager, Animal Health, Welfare and Biosecurity, Meat & Livestock Australia

Abstract/Resumen

Ticks impair profitable cattle production through their sanguiverous parasitism, skin and hide damage by bite wounds (exacerbated by secondary bacterial infection and/or flystrike), intoxication, and/or disease transmission. These different paths of pathogenicity imply that different management options might exist under specific circumstances. Under some conditions, host resilience might suffice for keeping tick numbers under control, but immunisation against tick-borne disease might be required. Where the use of chemicals for tick control is unavoidable, a predominance of multi-host ticks necessitates more complete cover, whereas intermittent application of chemicals is feasible where the main problem is a single-host tick. Using a vaccine against ticks on cattle is easier in smaller scale operations, than under extensive conditions, where cattle are mustered only once a year. The author will compare experiences in controlling ticks on cattle in southern Africa and Australia to illustrate these differences

From tick biology to the rational design of novel ‘anti-tick‘ strategies

Petr Kopáček, Daniel Sojka, Ondřej Hajdušek, Radek Šíma, and Jan Perner

Email: kopajz@paru.cas.cz
Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

Abstract/Resumen

Ticks are ectoparasitic mites completely dependent on the host blood as their exclusive source of nutrients. A striking trait of these blood-feeders is their extreme gluttony. Tick adaptations to their parasitic life-style resulted in major physiological departures from their hosts that intuitively serve as potential targets for efficient tick control. Therefore, our long-term research has been focused on the specific physiological processes associated with blood meal processing in ticks, e.g. intracellular blood digestion based on the network of cysteine and aspartic peptidases, heme auxotrophy, or iron metabolism.
The functional genomics based on RNA-interference substantially contributed to our knowledge of function of many tick molecules involved in blood meal processing and heme and iron metabolism. On the other hand, RNAi also revealed a great resilience of these physiological processes given the high redundancy of molecules involved in these systems. Another promising tool allowing us to address yet experimentally inaccessible biological questions in tick physiology is based on recently implemented technique of tick in vitro feeding. Using membrane feeding, we can dissect out other essential non-protein nutritional components of blood meal diet (e.g., heme, lipids, sugars etc.) and investigate their importance in the tick development, and reproduction. The new discoveries in the tick physiology achieved by *in vitro* feeding may lead to the identification of novel potential targets for the rational development of efficient preparations and/or vaccines protecting against ticks and diseases they transmit.

Acknowledgement: Supported by GACR 18-01832S and 19-04301S

Skin microbiota in outcomes of tick infestations: implications for development of immunobiological, semiochemical and probiotic interventions to reduce tick loads in cattle.

Isabel Kinney Ferreira de Miranda Santos

Email: imsantos@fmrp.usp.br
Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brasil

Abstract/Resumen

The author would address:

a. Current knowledge on immunity of skin and its appendages that pertain to ectoparasites

b. Current knowledge on the role of microbiota in regulating immunophysiology of skin and its appendages

c. Current knowledge on the role of ectoparasites in regulating skin microbiota

d. Current knowledge on the role of skin microbiota in regulating behavior of hematophagous and histiophagous parasites

e. Current knowledge on the role of microbial interactions in skin

f. Current knowledge on the role of host genetics in forming microbiota

Development of a tick vaccine with improved efficacy against *Rhipicephalus microplus* ticks

Th. Schetters[1,2], M. Madder[1,2], L. van den Berg[1], A. Evans[3], L. Meijer[3], J. Fourie[3]

Email: th.schetters@protactivity.com

[1]Clinglobal Ruisseau Creole, La Mivoie, Black River, Mauritius; [2]Department of Veterinary Tropical Diseases, University of Pretoria, South Africa; [3]Clinvet, Bloemfontein, South Africa

Abstract/Resumen

Isolates of *Rhipicephalus microplus* ticks from the different geographical regions show wide spread resistance to α-cypermethrin, chlorfenvinphos, and amitraz. As an alternative control measure of *R. microplus*, calves can be vaccinated with tick antigens that evoke protective immunity. Two vaccines based on the recombinant Bm86 protein have been commercialised (TickGard® and Gavac™). Data from ten-year use of these vaccines in the field showed that efficacy was on average a 50-60% reduction in the number of engorged adult female ticks. To improve the efficacy of the Bm86-based tick vaccines, the development of a vaccine with an additional tick antigen is being pursued. Initial studies indicated that immunization of calves with Bm86 and subolesin improved the level of immunity. Here we present additional results on the efficacy of immunization of cross-bred *Bos taurus* calves against Bm86 and an additional tick antigen (subolesin, ferritin-2 or aquaporin) formulated in Montanide ISA50V2 adjuvant. As control, groups of calves were vaccinated with either of these proteins alone. The dose was 50-100µg of recombinant protein in a volume of 2ml, and vaccinations were given by subcutaneous injection, three times, with at least a four-week interval. Two weeks after the final vaccination, calves were housed in individual stanchions and subsequently infested with *R. microplus* larvae from South-Africa using the whole body infestation method. Results showed that vaccination against Bm86 only did not induce significant protection against *R. microplus* infestation, neither did vaccination with any of the other tick proteins. Importantly, when calves were immunized with Bm86 and also with ferritin-2, significant reduction in the number of adult female ticks that dropped from the calves after engorgement was found (35%; p=0.03). The fact that this form of Bm86 did not induce protection (it was produced using a proprietary expression system of a third partner) will be further investigated.

Tick immunological control as a feasible alternative in field conditions.

Alina Rodríguez Mallon, Pedro E. Encinosa Guzmán, Yamil Bello Soto, Llilian Gómez Pérez, Ana Laura Cano Argüelles, Yuselys García Martínez, Marisdania Joglar Piñeiro, Frank Luis Ledesma and Mario Pablo Estrada García.

E-mail: alina.rodriguez@cigb.edu.cu
Center for Genetic Engineering and Biotechnology. P.O. Box 6162, Habana 10600, Cuba.

Abstract/Resumen

There are increasingly frequent reports of multi resistant tick strains to chemical acaricides. In this current situation, vaccination becomes in a very attractive alternative to control these ectoparasites. The challenge of research community working on anti - tick vaccines is to get effective antigens with a broad action spectrum. Bm86 vaccination has demonstrated to be effective against *Rhipicephalus microplus* ticks in field conditions when used as part of an integrated management strategy. The most important impacts of these Programs applied in Cuba and other countries are the reduction in tick infestations after some generations of ticks feed on vaccinated animals, diminution in the incidence of hemoparasitic diseases and a dramatic reduction in the use of chemicals. However, obtaining new effective antigens to be used alone or combined with Bm86 becomes of great relevance in order to improve the practical application of vaccines in the fight against other tick species. In this way, although a successful proof of concept in laboratory conditions with a new antigen is necessary, it is not enough in order to have a vaccine against ticks for field application. A development pathway should be performed in which effective formulations with technical and economic feasibility should be found. In addition, the scale up production process of the vaccine and the development of a robust analytic system to guarantee the effective vaccine quality control should be also addressed before assuming clinical trials for the sanitary register validation of the vaccine.

Acknowledgement: Some authors belong to the CYTED Network INCOGARR (118RT0541)

Vaccines candidates against ticks based on the conjugation of P0 peptide to three carrier proteins

González LJ[1], Rodríguez-Mallón A[2], Pouza S[1], Cabrera G[1], Espinosa LA[1], Besada V[1], Cabrales A[3], Diago D[3], Garay H[3], Guirola O[4], Vieyto JC[4], Jiménez S[5], Licea-Navarro A[5], Portela M[6], Leiva A[6], Durán R[6], Wiśniewski JR[7], Okumura N[8], Takao T[8], and Estrada MP[2].

Email: luis.javier@cigb.edu.cu

[1]Department of Proteomics, [2]Animal Biotechnology, [3]Peptide Synthesis, [4]Bioinformatics Department, from the Center for Genetic Engineering and Biotechnology, Havana, Cuba. [5]Departament of Biomedical Innovation, CICESE, Mexico. [6]Proteomics and Analytical Unit, Pasteur Institute, Montevideo Uruguay, [7]Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Berlin, [8]Germany and the Institute of Protein Research, Osaka University, Japan.

Abstract/Resumen

Ticks and ticks-borne diseases represent a threat to the animal and human health. The chemical conjugation of a peptide from P0 acidic ribosomal protein (pP0) from *Rhipicephalus* genus ticks to KLH has been proposed as broad coverage vaccine against ticks. An efficacy around 90% has been obtained when rabbits, dogs and cattle immunized with this conjugate have been challenged with *R. sanguineus*, *R. microplus* and *Amblyomma mixtum* ticks. P0 peptide with a Cys residue located at the N-terminal (Cys^1pP0) was also conjugated to the lysine residues of two carrier proteins: p64K from *Neisseria meningitidis*, and Bm86 antigen from *R. microplus* ticks using the same BMPS chemistry to generate the conjugates Bm86-Cys^1pP0 and p64K-Cys^1pP0. A fourth conjugate (p64K-βAla^1pP0) was synthetized by a single step reaction between the six free cysteine residues of p64K protein and the Mal-βAla^1pP0 peptide containing a maleimide group and a β-Ala^1 spacer at the N-terminal end. SDS-PAGE analysis revealed that conjugates Bm86-Cys^1pP0 and p64K-Cys^1pP0 are highly heterogeneous in size, carrying an average of nine and seven units of Cys^1pP0, respectively while KLH-Cys^1pP0 remained in the staking gel impairing the analysis of the molecular weight distribution. SDS-PAGE analysis of p64K-βAla^1pP0 revealed the presence of a highly homogeneous conjugate showing a single band at 96 kDa corresponding to the addition of six βAla^1pP0 units. All these conjugates were digested with several specific proteases and analyzed by LC-MS/MS. More than 90 % of the conjugation sites were coincidently assigned by three software (pLink, StavroX and Kojak) through the identification of type-2 cross-linked peptides. The MS/MS spectra were manually validated considering the presence of diagnostic ions that revealed the size of the cross-linked peptides. All lysine residues in Cys^1pP0 conjugates were found partially conjugated. Combination of SCX and MALDI-MS analysis yields a fingerprint for the cross-linked peptides that could be useful to evaluate the reproducibility of the conjugation procedure.

Understanding tick sialome towards vector and pathogen control

Ana Domingos[a,c], Joana Couto[a,c], Joana Ferrolhoa,[c], Gustavo S. Sanches[a,c], Margarita Villar[b], José de la Fuente[b,d], Sandra Antunesa,[c]

Email: adomingos@ihmt.unl.pt

[a]Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira, 100, 1349-008 Lisboa, Portugal. [b]SaBio. Instituto de Investigación en Recursos Cinegéticos IREC-CSIC-UCLM-JCCM, Ronda de Toledo s/n, 13005 Ciudad Real, Spain. [c]Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (GHTM-IHMT-UNL), Rua da Junqueira, 100, 1349-008 Lisboa, Portugal. [d]Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA

Abstract/Resumen

Babesiosis is a worldwide tick-borne disease caused by intra-erythrocytic parasites of the genus *Babesia* affecting a large variety of animals, including cattle, and humans. *Rhipicephalus* spp. are recognized as the main vectors and reservoirs of different *Babesia* species showing a significant negative impact on animal and human health. Our studies were focused in the two systems *R. annulatus – B. bigemina* and *R. bursa - B. ovis* that causes important losses in both cattle and small ruminant production. Tick salivary glands (SGs) are morphologically complex organs with multifunctional roles in different biological processes such as osmoregulation, feeding and pathogen transmission. Understanding the SG molecular dynamics is a key for the discovery of pharmacologically active compounds of clinical interest such as protective antigens for anti-tick and pathogen transmission blocking vaccines. Experiments were designed to combine data from omics technologies with gene silencing aiming to find new insights into the molecular interplay occurring at the tick-pathogen interface allowing the selection of genes/proteins related to the infection process.

SGs from uninfected *Rhipicephalus* and *Babesia* infected ticks were obtained. RNA and proteins were isolated for either transcriptomics or proteomics analysis using RNA sequencing (RNA-seq) and reverse phase liquid chromatography coupled with mass spectrometry (RP-LC-MS/MS). Obtained transcripts and proteins were functionally annotated and the differences on gene/protein expression and representation in the two tick-parasite systems were analyzed. *In vivo*, gene functional analysis was carried on by RNAi-mediated gene silencing, targeting selected transcripts/proteins in order to validate their impact in infection and vector biology. The role of genes on tick reproductive parameters and interaction with *Babesia* infection was evaluated. Our results suggest a strong parasitic pressure over tick cellular functions highlighting molecules such as UB2N and lachesin as important players in the response to pathogen infection.

Acknowledgement: Speaker participation in BioTicks 2019 was supported by CYTED Network INCOGARR (118RT0541)

In silico analysis of proteins from salivary glands of *Amblyomma cajennense* for the identification of conserved antigens.

Merino CJO[1*].De la Cruz HNI[1], Lagunes QRE[2], Romero SD[4], De La Fuente GJ[3]

Email: mero840125@hotmail.com
[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Km. 5, Carretera Victoria-Mante, Cd. Victoria, Tam., México. CP 87000.
[2]Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria, INIFAP. AP 206, Civac, Jiutepec, Mor., México. CP 62550.
[3]SaBio. Instituto de Investigación en Recursos Cinegéticos IREC-CSIC-UCLM-JCCM, Ronda de Toledo s/n, 13005 Ciudad Real, España.
[4]Laboratorio de Parasitología. UD PZTM. Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, Veracruz, Mexico.
*Responsable del trabajo: mero840125@hotmail.com

Abstract/Resumen

Ticks are arthropod vectors of pathogens affecting human and animal health as well as animal welfare and production worldwide. After the cattle tick Rhipicephalus microplus) the ticks of the Amblyomma genera are the most important blood sucking parasite of a variety of livestock species with an impact on cattle industry in tropical and subtropical regions of the world. The main method of control of ticks has been the usage of chemical products; however, the use of acaricides has resulted in some serious drawbacks such as acaricide-resistant ticks and environmental pollution. As a result the use of immunological control using tick proteins is suggested as an alternative to control tick populations; however the use of this antigens have been limited due to several differences in the immunological response in different tick species, therefore In this research we identified proteins that are present in the Salivary gland of A. cajennense by a proteomic analysis in order to identify new conserved antigens that help to control tick infestations. For this purpose, our team got tissues of A. cajennense then all proteins were extracted, purified and quantified. In order to identify them, proteins were digested and analyzed by RP-LC–MS/MS. Once identified, ontology was carried out to characterize the biological process, molecular function and cellular component of each protein. Results showed that proteomic analysis allowed the identification of 1,392 and 1947 membrane and cytoplasm proteins, respectively, from these, proteins associated to metabolic process resulted as the most important function due to the amount of proteins identified. In conclusion this research is aimed to improve tick vaccine efficacy and safety by combining protective antigens from different ticks and information provided will increase the quality of anti-tick vaccines.

From transcriptome analysis of *Ixodes ricinus* synganglion to functional characterization of neuroreceptors

Olivier Plantard[1], Caroline Hervet[1], Nathalie de la Cotte[1], Claude L. Charvet[2], Clément Auger[2], Nollan Puget[2], Nicolas Lamassiaude[2], Elise Courtot[2], Cédric Neveu[2], Steeve Thany[3], and Claude Rispe[1]

Email: olivier.plantard@inra.fr
[1] BIOEPAR, INRA, Oniris, Nantes, France
[2] ISP, INRA, Université Tours, Nouzilly, France
[3] LBLGC, Université d'Orléans, Orléans, France

Abstract/Resumen

To control tick populations, chemical control remains the most commonly used method. However, acaricide resistances have been reported against all acaricide drug classes developed to date. Moreover, because they are all targeting neuroreceptors found both in ticks and in other groups of arthropods, acaricides are responsible for negative side effects on the entomofauna. There is thus an urgent need to develop new acaricides being more selective and more durable. Those new acaricides should act on targets more specific to ticks and with low polymorphism within populations.

In the aim of identifying such targets, taking advantage to large genomic and transcriptomic resources available for this species, we performed the transcriptome analysis of Ixodes ricinus synganglion to get the full-length sequences of all its neuroreceptor genes. We achieved a complete catalogue of the Cys-loop Ligand-Gated Ion Channel genes, including the gamma-aminobutyric acid-gated channels (GABACl) and the nicotinic acetylcholine receptors (nAChRs). Some sequence polymorphisms were observed within natural tick populations suggesting that resistances may arise quickly after acaricides use.

Firstly, we performed two-electrode voltage clamp recordings after micro-injection in Xenopus oocytes of in vitro synthesized cRNAs encoding the IriRDL subunit. Perfusion of GABA induced an inward current demonstrating that IriRDL subunit formed a functional homomeric GABACl. The acaricides fipronil and lotilaner potently inhibited the effect of GABA.

Secundly, we developed a microtransplantation method where whole membranes were extracted from neurons of tick synganglion and injected into Xenopus oocytes. The presence of functional nAChR was demonstrated as well as their susceptibility to several neonicotinoids.

This genome-to-lead approach allowed the successful identification of targets for the development of acaricides that can be investigated by heterologous expression or membrane transplantation. Those tools pave the way to conduct high throughput screening to isolate new acaricides, more selective and more durable to control ticks and tick-borne diseases

Control of *Ichthyophthirius multifiliis* (Ich) by mucosal immunoglobulins: Novel findings and implications for the design of effective vaccines against Ich.

J. Oriol Sunyer

Email: sunyer@vet.upenn.edu

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract/Resumen

Ichthyophthirius multifiliis (Ich) is an ectoparasite that infects a wide variety of freshwater fish. Ich causes a disease commonly known as white spot disease, which results in significant economic losses to freshwater aquaculture. Until recently it was unknown the immune mechanisms by which fish fight Ich, and this lack of knowledge has thwarted the search for effective immunotherapeutics, including vaccines. A few years ago our group discovered the existence of a previously uncharacterized mucosal immunoglobulin (IgT) in teleost fish. We have shown that IgT plays a key immune role in several fish mucosal surfaces, including the gills, skin, gut, and buccal cavity. More specifically, we have demonstrated that several mucosal pathogens, including Ich, induce significant pathogen-specific IgT titers in all studied mucosal surfaces, whereas IgM-specific titers are for the most part confined to systemic sites. Moreover, in all mucosal surfaces tested, IgT is the prevalent immunoglobulin coating the microbiota, akin to mucosal IgA in mammals. To definitely demonstrate the key role of IgT in fighting Ich infections, we generated an IgT-depletion fish model in which IgT and the B cells producing it where temporarily depleted for an 8 week period. Upon IgT depletion, fish became significantly more susceptible to Ich infection, as seen by the dramatic increases in the parasite loads of IgT-depleted fish as well as their increased mortality rates. Thus far, the fish farm industry lacks vaccines against Ich, and treats Ich infections with a variety of chemotherapeutics. Taking advantage of the knowledge showing the critical contribution of IgT against Ich infections, we have started developing vaccine strategies that induce significant mucosal IgT responses against this parasite. Recent data shows the development of a promising mucosal vaccination strategy that induces strong IgT titers, and protects fish from Ich.

Vaccines against sea lice, a promising alternative but still challenging

Yamila Carpio.

E-mail: yamila.carpio@cigb.edu.cu
Center for Genetic Engineering and Biotechnology. P.O. Box 6162, Habana 10600, Cuba.

Abstract/Resumen

Losses due to salmon lice are limiting Atlantic salmon's aquaculture growth and compromising its sustainability. Costs are set to increase as there is not effective vaccine against salmon lice. Only a small number of anti-parasitic drugs are currently licensed for treatment. These drugs are losing their efficacy due to evolved parasite resistance. Although emerging sea lice proteins have been identified recently as potential targets for generating protective molecules, only a limited number of them have been evaluated in vaccine trials with unsuccessful results. In this context we have identified two concealed antigens from *Lepeophtheirus salmonis*, the subolesin/akirin/my32 and ribosomal protein P0, which are involved in the control of critical parasite developmental processes. Different vaccine candidates based on these proteins were produced in *Escherichia coli* and their efficacy tested. We were able to successfully demonstrate initial proof of concept which provides more comprehension for vaccine approaches and for the interpretation of results from lice challenge experiments under laboratory controlled environment. The logical next steps in the development of a commercial vaccine would be field trials to test the impact in the reduction of chemicals use and validate the efficacy of the vaccine in the context of integrated control programs.

Manejo integral de los tratamientos garrapaticidas.

Dr. Eduardo Ramírez

E-mail: eduardo.ramirez@bayer.com
Gerente Nacional Técnico Bovinos de pastoreo, Bayer de Mexico

Abstract/Resumen

En Bayer, sanidad animal, como parte de la Comisión Nacional de Parasiticidas (INFARVET), nos enfocamos a promover el buen manejo de los diferentes plaguicidas, utilizados principalmente para el control de ectoparásitos, tratamos de llegar de manera directa a los usuarios mediante diferentes fuentes de información. A pesar de toda la información que se maneja sobre control de garrapatas en bovinos, se siguen presentado infestaciones graves en las unidades de producción lo que conlleva a pérdidas económicas por año de casi 574 millones de dólares americanos (Rodríguez Vivas). Múltiples factores se unen en la problemática, incluidas la falta de información al productor sobre el buen manejo de las diferentes moléculas garrapaticidas y aprobadas en México, asesorías en la materia, las cuales no siempre son las más correctas, regulaciones gubernamentales de productos para el control de garrapatas (México), resistencia de Rhipicephalus microplus a diferentes ingredientes activos, etc. Necesitamos capacitar a más y más personas en el buen manejo de los tratamientos garrapaticidas, sin dejar a un lado el costo–beneficio para el productor, a fin de evitar al máximo el desarrollo de resistencia por parte de las garrapatas. Hoy día tenemos un arsenal completo de moléculas para control de garrapatas y métodos de aplicación, esto no es suficiente si no se acompaña de programas que realmente resuelvan las necesidades de los productores. Dichos programas deben tener las siguientes características: servicio, confianza, honestidad, moléculas y productos autorizados (CENAPA-SENASICA) y buen manejo de productos químicos. Todo ello, dentro de un manejo integral que permita la inclusión de familias químicas con diferente mecanismo de acción, rotación de moléculas, manejo del umbral económico, manejo de pastizales, así como la utilización de métodos alternativos, sin dejar de lado las buenas prácticas sanitarias que lleven al cuidado y protección de nuestro medio ambiente y sustentabilidad necesaria para toda práctica pecuaria no solo en México si no en el Mundo entero.

Experiencias en el uso de una vacuna recombinante de Bm86 contra la garrapata *Rhipicephalus microplus* en México.

Ortiz EM[1]*, Soberanes CN[1], Lagunes QR[2]

E-mail: martin.ortiz@lapisa.com
[1]LAPISA. Carretera La Piedad-Guadalajara km 5.5, Colonia Camelinas. C.P. 59375, La Piedad, Michoacán, México. [2]CENID-SAI, INIFAP. Carretera Federal Cuernavaca-Cuautla #8534, Colonia Progreso. Jiutepec, Morelos, México.

Abstract/Resumen

Las infestaciones por garrapatas *R. microplus* producen un impacto económico en la ganadería bovina, reduciendo la ganancia de peso y la producción de leche. Así mismo, son capaces de transmitir patógenos que causan Babesiosis y Anaplasmosis. El control se centra principalmente en la utilización de ixodicidas. Sin embargo, el uso indiscriminado de estos productos ha generado la selección de poblaciones de garrapatas resistentes a diferentes acaricidas comerciales, por lo cual se requieren nuevas alternativas de control como las vacunas. El objetivo del trabajo consiste en constatar la efectividad biológica de la vacuna Bm86 en bovinos naturalmente infestados con garrapatas *R. microplus*, dentro de un programa de control integrado. En los cinco ensayos realizados, los resultados obtenidos son similares, se observa una disminución de garrapatas *R. microplus* en los hatos vacunados con Bm86. Los intervalos entre los baños garrapaticidas, son más espaciados en la medida que transcurre el tiempo de la aplicación del programa. En ninguno de los casos hubo presencia de animales enfermos por hemoparásitos. El ahorro en el recurso baño fue significativo en los cinco ensayos ya que el intervalo de baño antes de los ensayos era de 15 días. Se obtuvo durante la primera prueba intervalos sin baño de 78 días, para la segunda de 90 y 113 días, y para la tercera 80 días. En cuanto a la infestación por garrapatas *R. microplus*, en los tres primeros ensayos fue significativo y los resultados de limpieza se vieron afectados por la poca susceptibilidad de los productos a las poblaciones de garrapatas de los ranchos, sin embargo, ente los días 80 a 100 posteriores a la tercera vacunación, los baños se alargaron significativamente. Finalmente, la utilización de la vacuna a base de Bm86, es prometedora en ranchos que presentan resistencia a casi todas las moléculas ixodicidas existentes; se debe manejar dentro de un programa de control integrado y el beneficio principal se obtiene en la reducción de la contaminación ambiental y de subproductos derivados como carne y leche en los animales vacunados.

CIGB collaborations with CAMEVET: Gavac, the first subunit immunogen registered in a document with a format approved for the Americas.

Jesús Mena, Hector Machado, Yandiesky Lowery et al. from CIGB and DSA, MINAG.

E-mail: jesus.mena@cigb.edu.cu
Center for Genetic Engineering and Biotechnology. P.O. Box 6162, Habana 10600, Cuba.

Abstract/Resumen

A summary of the results of more than 12 years of the sustained collaboration of the CIGB with the OIE regional organization (CAMEVET) is presented. The collaboration has been carried out in close coordination with the Focal Point of Cuba: "The Veterinary Medicines Registry Office of the Animal Health Directorate". As a main result, the regional approval of the "REGISTRATION FORM FOR SUBUNIT IMMUNOGENS OBTAINED THROUGH BIOTECHNOLOGICAL PROCESSES" was achieved. This guideline has already been implemented and used for Gavac registration in several countries of the region. More recently, work has been done on the preparation of the “General Guidance for Test Kits Intended for the Diagnosis of Animal Diseases”.

High-throughput nanotechnologies for tick-borne pathogens detection

Sara Moutailler

Email: Sara.MOUTAILLER@anses.fr

Groupe Vectotiq.UMR BIPAR, ANSES, INRA, ENVA, Université Paris-Est ANSES - Laboratoire de Santé Animale France. email : sara.moutailler@anses.fr

Abstract/Resumen

Worldwide, ticks transmit more pathogens than other arthropods (around 60 bacteria, 30 parasites and 100 viruses; a third of them are responsible for zoonosis). Due to increased travel, climatic, and environmental changes, the incidence of tick-borne disease in both humans and animals is increasing throughout the world. Therefore, extended surveillance tools are desirable to better control ticks and tick-borne pathogens transmitted. To accurately screen tick-borne pathogens, new epidemiological tools were implemented in order to identify/detect 65 bacteria, 6 bacteria genus, 28 parasite species, 53 viruses and 8 tick species. Then large scale epidemiological studies were conducted through collaborative projects at the international level first in hard ticks, then in soft ticks and finally in mammals. Those advanced methodologies permitted the detection and the estimation of prevalence of expected, unexpected and rare tick-borne pathogens in different countries. Those new tools also demonstrated their ability to study tick co-infection and genetic diversity of tick-borne pathogens. Those surveillance methods represent a major improvement in epidemiological studies, able to facilitate comprehensive testing of tick-borne pathogens in ticks, mammals and humans, and which can also be customized to monitor emerging diseases.

Resistance of tick microbiota to biological disturbance.

Alejandro Cabezas Cruz

Email: cabezasalejandrocruz@gmail.com

Department of Animal Health. National Institute for Agricultural Research, INRA, France.

Abstract/Resumen

Ixodes scapularis ticks harbor microbial communities including pathogenic and non-pathogenic microbes. Pathogen infection increases the expression of several tick gut genes, which disturb the tick gut microbiota and impact bacterial biofilm formation. Here, we tested the resistance (insensitivity to disturbance) of tick microbiota and tick microbiome to pathogen infection, antimicrobial peptides and anti-tick immunity. We demonstrated that *Anaplasma phagocytophilum* infection and the tick antimicrobial peptide IAFGP have small impact on the taxonomic and functional traits of tick microbiota. In contrast to pathogen infection and antimicrobial peptide, host immunity specific to the tick protein PIXR disturb dramatically the tick microbiota and the composition and abundance of metabolic pathways in the *I. scapularis* metagenome. Anti-tick immunity increases the representation and importance of polysaccharide and siderophore biosynthesis pathways involved in biofilm formation while these pathways are under-represented in the gut microbiome of ticks infected by *A. phagocytophilum* or exposed to IAFGP. These analyses reveal that tick microbiota in highly sensitive to anti-tick immunity and resistant to pathogen infection and antimicrobial peptides. The formation of dense biofilms due to increase in biofilm formers may be part of a protective response of tick microbiota to anti-tick immunity. This raises an interesting question; can anti-tick vaccination trigger the formation of biofilms that increase the resistance of ticks to anti-tick vaccines?

An integrated approach for the molecular identification of ticks and detection of tick-borne diseases

Luis M. Hernández-Triana[1*], Kayleigh M. Hansford[2], Paul Phipps[1], Mar Fernández de Marco[1], Emma L. Gillingham[2], Liz McGinley[2], Ben Cull[2], Jolyon Medlock[2], Nadya I. Nikolova[3], Lorraine McElhinney[1], Nicholas Johnson[1] and Anthony R. Fooks[1]

[1]Animal and Plant Health Agency, Virology Department, Woodham Lane, Addlestone, Surrey, United Kingdom. [2]Medical Entomology and Zoonoses Ecology, Emergency Response, Department, Public Health England, Porton Down SP4 0JG. [3]Centre for Biodiversity Genomics, University of Guelph, Guelph, Ontario, Canada. *Corresponding and presenting autor: Luis M. Hernández-Triana, email: luis.hernandez-triana@apha.gov.uk

Abstract/Resumen

Ticks transmit numerous pathogens to humans and livestock, and tick-borne diseases are currently considered a hindrance to economic development worldwide. There are 23 species recorded in the United Kingdom, although exotic species such as *Rhipicephalus sanguineus* s.l. and *Hyalomma marginatum* are sometime found on imported animals. The ability to quickly and efficiently confirm the species of tick and identify associated pathogens such as piroplasms, bacteria and viruses is useful in the surveillance and control of tick-borne disease. In this study, we have developed an integrated pipeline of tests to achieve this and applied it to tick samples from the UK. Nucleic acid extraction was carried out, then COI DNA barcoding PCR was applied for the molecular identification of ticks, and generic or specific qPCRs were employed for the detection of pathogens. The DNA barcode analysis of 540 sequences from 22 morphologically assigned species revealed that most specimens grouped as predicted with an average intraspecific genetic diversity of 2.6%. However, *R. sanguineus* s.l. separated into three sub-clades with high genetic diversity (3.8%). PCR for the detection of piroplasms revealed the presence of *Babesia canis* in *Dermacentor reticulatus*, while cases of suspected mortality in livestock caused by ticks revealed *Theileria luwenshuni* in sheep. *Borrelia burgdorferi* has been found repeatedly in *Ixodes ricinus*, while species of *Rickettsia*, *Babesia* and *Ehrlichia* were found in the bat tick *Carios vespertilionis*. Because of the recent discovery of *Hyalomma rufipes* in the UK, one of the main vectors of Crimean-Congo fever virus, we tested for the presence of this and other and pathogens. No viruses were detected, although the tick tested positive for *Rickettsia aeschlimannii*. In conclusion, by generating a barcode library in combination with qPCRs for the detection of pathogens, this study confirms the effectiveness of this approach for the detection of pathogens in tick populations.

Current Fever Tick Research in Texas

Dee Ellis DVM MPA

Email: Dee.Ellis@ag.tamu.edu

Institute for Infectious Animal Diseases (IIAD), Texas A&M University System, USA

Abstract/Resumen

Dr. Dee Ellis of the Texas A&M Institute for Infectious Animal Diseases in College Station, Texas, will discuss the ongoing Cattle Fever Tick Vaccine research projects currently under way by Texas A&M University scientists and other partners in Texas. The current research initiatives include projects for vaccine development, novel antigen validation, and effective treatments for cattle and wildlife. Research underway also includes evaluations of susceptible population densities or movements, habitats and ecosystems (Big Data). The talk will also review strategies used by Texas A&M scientists, industry stakeholders and government regulators to pre-identify priority research needs. The result when desirable research needs are pre-identified and agreed upon by all parties is that the proposed projects have a better chance of approval by funding entities.

Severe fever with thrombocytopenia syndrome virus from ticks and animals

Sun-Woo Han[1], Yoon-Kyoung Cho[1], Jun-Gu Kang[1], Kyoung-Seong Choi[2], Joon-Seok Chae[1], *

[1]Laboratory of Veterinary Internal Medicine, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Republic of Korea. [2]Department of Animal Science and Biotechnology, College of Ecology and Environmental Science, Kyungpook National University, Sangju, Gyungsangbuk-do 37224, Republic of Korea. *Corresponding author. Email: jschae@snu.ac.kr

Abstract/Resumen

Severe fever with thrombocytopenia syndrome (SFTS) is caused by a novel tick-borne Huaiyangshan banyangvirus in the Phenuiviridae family in China, the Republic of Korea (ROK), Japan, and Vietnam. SFTS is mainly characterized by fever, leukopenia and thrombocytopenia in human and few animals. The purpose of this study is to investigate the SFTS virus (SFTSV) infection in tick vector and animal host in the ROK. To investigate the prevalence of SFTSV from ticks and animals in the ROK, ticks were collected from 5 national parks in 2015, and animal sera were collected from domestic, wild and companion animals during 2013-2019 in the ROK. Total collected ticks were 1,470 ticks, 1,061 nymph and 409 adult ticks in 5 national parks. Also, sera were collected from total 2,530 animals. Viral RNA was extracted from ticks and sera using viral RNA extraction kit. One-step RT-nested PCR was performed to amplify the S segment of the SFTS virus. The sequence data were analyzed using Chromas and were aligned using CLUSTAL X. The phylogenetic analysis was constructed using the neighbor-joining method in MEGA7.The total infection rate of SFTSV was 3.6% in ticks (*Haemaphysalis longicornis, H. flava, Ixodes nipponenesis*, and *Amblyomma testudinarium*), 2.6% in nymphal and 6.1% in adult ticks. The total infection rate of SFTSV was 3.7% in animals. Thirty two of 1,005 (3.2%) goats, 4 of 240 (1.7%) domesticated pigs and 12 of 99 (12.1%) cattle, 3 of 264 (1.1%) dogs, 33 of 354 (9.32%) cats and 6 of 493 (1.2%) horses, 1 of 21 (4.8%) Korean water deer, and 2 of 54 (3.7%) wild boars were positive for SFTSV. Based on phylogenetic analysis, SFTSV is generally classified into Japanese and Chinese clades. These results are indicates that SFTS virus may circulated in domestic, companion and wild animals in in natural environments.

Genetic diversity of *Anaplasma marginale* in cattle from Itú, state of São Paulo, southeastern Brazil

Garcia, A. B., Jusi, M. M. G., Freschi, C. R., Ramos, I. A. S.; Mendes, N. S.; Amaral, R.B.; André, M. R.; Machado, R. Z.

Email: rzacariasmachado@gmail.com
Facultad de Ciencias Agrarias y Veterinarias. Universidad Estadual Paulista (FCAV-UNESP), Sao Paulo, Brasil

Abstract/resumen

Anaplasma marginale, an obligate intracellular erythrocyte bacterium, is the main agent of bovine Anaplasmosis, a disease that affects cattle herds, causing severe anemia, weight and milk production lossses and, consequently, generating large economic losses in livestock worlwide. Transmission is mediated by biological vector (ticks), mechanically by blood-sucking flies, transplacentally or fomites with infected blood. The genetic diversity of this bacterium has been characterized based on the surface protein sequence (MSPs), mainly the MSP1α protein. The present study aimed to verify the genetic diversity of *Anaplasma marginale* in Angus beef cattle naturally infected during an outbreak of the disease. The cattle was maintained in a herd, from a farm in the city of Itú, state of São Paulo. A total of 80 blood samples were obtained and submitted to DNA extraction, indirect enzyme immunoassay (iELISA), quantitative PCR (qPCR) for *msp1β* gene, semi nested PCR (snPCR) for *msp1α* gene. Positive samples in nPCR assays were cloned and submitted to Sanger sequencing followed by genetic diversity analysis by RepeatAnalyzer software. The Indirect Immunoenzymatic Assay (iELISA) revealed the presence of antibodies IgG *anti-A. marginale* in 22.5% of the sampled animals. In the qPCR, 100% of the samples were positive, with quantification ranging from 10^3 and 10^5 *msp1β* copies/µL. In the semi-nested PCR, based on the *msp1α* gene, 57.5% (46/80) of the samples were positive. Microsatellite analysis of 36 sequences obtained showed the presence of genotypes H (58.3%), F (25%), E (19.4%), and a sample with genotypes C and a G. Strains of thirty-six non-cloned samples and twelve cloned samples were identified in the region studied, with several strains never previously described in the literature, such as *13 27 13 27 13 F*; *16 F F*; *τ 27*; *63 29 104 29*; *LJ1 13 LJ1 13*; *16 F 17*; *16 F 91*, among others. High genetic diversity of *A. marginale* bacteria was found in this farm located in Itú, São Paulo. It was concluded from this study the existence of high genetic diversity of *Anaplasma marginale* in the sampled cattle of Angus breed, from Itu-SP.

Keywords: iELISA, PCR, *msp1α* gene, *msp1 β*

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Situation of bovine hemoparasitosis transmitted by *Rhipicephalus microplus* in Cuba.

R. Rodríguez Fernández; L. Méndez Mellor; A. Fuentes Castillo and Y. Hernández Rodríguez

Laboratorio Nacional de Parasitología, ULCSA, Ministerio de la Agricultura, Cuba.
Email: rafmary.rodriguez@lnp.art.minag.cu

Abstract/Resumen

The hemoparasites transmitted by *Rhipicephalus microplus* ticks, which affect cattle in Cuba, are *Babesia bovis, Babesia bigemina* and *Anaplasma marginale*. In the 90's of the last century, these hemoparasitosis constituted an important cause of bovine losses and therefore many economic damages for the Cuban cattle livestook. The objective of this work is to expose the behavior of bovine hemoparasitosis in Cuba during the last 28 years. The official reports of the epizootiological indicators (outbreaks, sick and dead) of Babesiosis and bovine Anaplasmosis, from 1990 to 2017, were taken from the National Directorate of Animal Health. As a result of the evaluation of these epizootiological indicators, it was possible to verify that the incidence of Babesiosis and Anaplasmosis in the analyzed period experienced a tendency to diminution of outbreak, sick and dead in relation to the reports in the first years of 90’s, although the report of Anaplasmosis always behaved superior in relation to Babesiosis during all years analyzed. The behavior of the incidence of these hemoparasitosis is influenced by the application in Cuba of the Program of Integrated Control of the Ticks (PICT) against *Rhipicephalus microplus*; In the case of the behavior of Anaplasmosis is due to other vectors are responsible for its propagation. We conclude that the tendency to decrease in our country the incidence of bovine hemoparasitosis transmitted by *Rhipicephalus microplus* is related to the implementation of a Program of Integrated Control against ticks.

Dynamics of genomic variations in *Leishmania panamensis* during the generation of resistance to trivalent antimonial.

Restrepo C.M. [1§], Llanes A. 1[§], Cedeño E.M. [2], Chang J.H. [2], Álvarez J. [3], Ríos M.[4], Penagos H. [5], Suárez J.A. [4], Lleonart R. [1]

Email: rlleonart@indicasat.org.pa

[1]Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), [2]Universidad Latina de Panamá, [3]Universidad de Panamá, [4]Instituto Conmemorativo GORGAS de Estudios de la Salud, [5]Caja del Seguro Social, Chiriquí. [§]Authors with similar contribution.

Abstract/Resumen

As in any other eukaryotic organism, the regulation of gene expression in *Leishmania* parasites is vital to assure proper functioning and survival in changing environmental conditions. However, in Trypanosomatids this regulation is not mainly at the rate of transcription initiation. Instead, posttranscriptional steps are finely tuned to change gene messenger stability and processing, and ultimately up- or down-regulate particular proteins when needed. Additionally, these organisms are able to quickly respond to environmental changes by selectively amplifying particular segments of the genome, increasing the gene dosage of particular sets of genes. In previous works, we had sequenced and characterized the genome of the *Leishmania panamensis* strain PCS-1, showing genomics variations which include changes in somy of particular chromosomes and the presence of an amplified lineal minichromosome derived from chromosome 34. In this work, we studied the genomic variations occurring during the generation of resistance to trivalent antimonial (SbIII) *in vitro*, and comparing them to those observed in recent field isolates. This compound is the active form of the main drug used in Panama to treat cutaneous leishmaniasis, the pentavalent antimonial. While effective, this treatment has important drawbacks, such as serious side effects and the generation of resistance in many parts of the world. We observed significant and progressive changes in the structure of the genome after stepwise exposure to increasing concentrations of the SbIII, including changes in somy at several chromosomes, amplifications of particular regions, and copy number variations at gene arrays. Strikingly, the appearance of certain potentially advantageous amplifications appears to be associated to the reduction of amplifications at other genomic regions, including the lineal minichromosome. These types of changes were not detected in the genomes of recent isolates from cutaneous leishmaniasis patients. In these parasites, the main genomic variations appear to be those generating gene arrays, where the number of genes is frequently larger than in laboratory propagated strains. This work contributes to the knowledge of how genome plasticity in *L. panamensis* contributes to the adaptation of the parasite to changing environment, including the generation of resistance to antimonials, the first line anti-leishmanial drugs used in Panama.

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Trade-off between allergy and protection to tick-borne diseases

Alejandro Cabezas Cruz

Email: cabezasalejandrocruz@gmail.com

Department of Animal Health, National Institute for Agricultural Research, INRA, France

Abstract/Resumen

Immunity to α-Gal provides a good model to study how antibodies to α-Gal might promote allergy and/or protection against pathogen transmission by vectors. Evidence suggests that individuals with blood type B produce fewer anti-α-Gal IgE Abs, and that Alpha-Gal syndrome (AGS) is strongly associated with blood type B negative individuals. The reduced capacity of blood group B individuals to produce anti-α-Gal Abs is presumably due to tolerance to α-Gal, which is similar to blood group B antigen. In agreement with the negative effect of blood group B on anti-α-Gal immunity, we discovered that the frequency of blood group B is positively correlated with the incidence of malaria in endemic regions. Interestingly, Lyme disease patients do not develop high anti-α-Gal IgE compared to AGS patients. High anti-α-Gal IgE in AGS patients correlates with high anti-α-Gal IgG. An interesting hypothesis emerges: AGS patients who are blood group B negative may produce high levels of anti-α-Gal IgG and IgM which protect them from Lyme disease. Hence a strong immune response to α-Gal may protect against Lyme disease with the trade-off of developing AGS.

The *Rhipicephalus sanguineus* complex: What's next?

Filipe Dantas-Torres

Instituto Aggeu Magalhães, Fundação Oswaldo Cruz (Fiocruz), Recife, Pernambuco, Brazil. email: filipe.dantas@cpqam.fiocruz.br

Abstract/Resumen

The *Rhipicephalus sanguineus* complex is a group of tick species, which includes *Rhipicephalus sanguineus* sensu stricto and some closely-related species. Some of these species are difficult to distinguish with certainty, due to their high pleomorphic nature and overlapping morphological features. Genetic studies have shed light into the systematic status of some species of this complex, including *R. sanguineus* s.s., whose neotype was recently designated. This taxonomic act paved the way for a better understanding of the *R. sanguineus* complex, not only from a taxonomic view, but also from a medico-veterinary standpoint. Further studies regarding the vector competence and insecticide susceptibility of species belonging to this group are needed.

Keywords: brown dog ticks; taxonomy; pathogen transmission; control.

Rhipicephalus sanguineus tropical and temperate lineage: a proteomic overview

Sanches GS,[a] Ferrolho J.[a] Couto J,[a] Bechara GH,[b] Machado RZ,[c] André MR,[c] Villar M,[d] de la Fuente J[d], Antunes S[a], Domingos A[a].

Email: gustavoseron@hotmail.com

[a] Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal; [b] Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná – PUCPR, Curitiba, Brazil; [c]Departamento de Patologia Veterinária, Faculdade de Ciências Agrárias e Veterinárias - FCAV, Universidade Estadual Paulista - UNESP, Jaboticabal, SP, Brasil; [d] SaBio. Instituto de Investigacion en Recursos Cinegeticos IREC-CSIC-UCLM-JCCM, Ciudad Real, Spain

Abstract/Resumen

For over 200 years *Rhipicephalus. sanguineus* sensu stricto was believed to be a single taxon, however, several studies have shown the existence of at least two genetic lineages under the name *R. sanguineus*: the temperate and the tropical lineages These lineages present strong differences in aspects related to morphology, biology and importantly vector competence for *Ehrlichia canis*. This bacterium is responsible for the canine monocytic ehrlichiosis, which infects monocytes and macrophages of wild carnivores and dogs. Keeping in mind this different vector competence of the lineages of *R. sanguineus*, the present study aimed to understand biological differences between both lineages, and also to elucidate how these lineages respond to *E. canis* infection in terms of protein representation. For this, the sialome and the mialome of the tropical versus the temperate lineage of *R. sanguineus* in non-infected and *E. canis* infected conditions were obtained by reversed phase liquid chromatography–mass spectrometry (RP-LC-MS/MS) and further analyzed. The functional characterization and classification of the proteins identified were carried out according to Gene Ontology hierarchy (GO), using the Blast2GO software. The results obtained suggest that the tropical lineage is metabolically more active than the temperate lineage that could be consequence of different evolutionary survival strategies which synchronize the life cycle with favorable abiotic conditions and availability of hosts. Concerning vectorial capacity, several cement related proteins were found exclusively in the infected salivary glands suggesting that cement cone has a role in pathogen transmission. The results obtained represent a step forward to increase our understanding about the mechanisms responsible for the different vector competence of both lineages of *R. sanguineus* to *E. canis* transmission and open avenues for the identification of molecules with potential to be used to the development of a vaccine targeting both tick fitness and tick-pathogen transmission.

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Current taxonomic status of the main ixodid ticks with veterinary and public health interest in Cuba

Pedro Encinosa Guzmán[1], Yamil Bello Soto[1], Yuselis García[1], Claudia Fernández[1], Daniela Aliaga[1], Yilian Fernández[2], Ananayla Vizcaino[2], Alier Fuentes[3], Rafmary Rodríguez[3], Hector Córdova[4], Ricardo L. Lleonart Cruz[5], Loreta García[4], Luis Méndez[3], Mario Pablo Estrada García[1] and Alina Rodríguez Mallon[1].

Email: pedro.encinosa@cigb.edu.cu

[1]Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology, Havana, Cuba. [2] Center for Advanced Studies of Cuba. [3] National Laboratory of Parasitology, MINAG, Cuba. [4] Flora y Fauna, MINAG, Cuba [5] Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), Panamá.

Ticks are ectoparasites widely distributed throughout the world and are found parasitizing a significant number of domestic and wild animals as well as humans. In Cuba, there are described nine species of ixodid ticks, four of which are considered important for Veterinary Medicine and Public Health. Three of these species, *Rhipicephalus sanguineus*, *Rhipicephalus microplus* and *Amblyomma cajennense* are involved in a worldwide taxonomic debate because they have morphological characteristics very similar to other species of the same genus that have been grouped as species complexes. Molecular biology advances have allowed the better understanding of the taxonomic status of each of them. In Cuba, well documented taxonomic studies of ticks are scarce. The objectives of this work focused on morphological and molecular characterization of these species present in the country. Scanning electron microscopy was used to thorough morphologic and morphometric studies and 16S, ITSII and COX I genes were used to approach the molecular taxonomic status. The results have shown that the analyzed field isolates belong to the *R. sanguineus sensu lato* species of the tropical lineage, to the *R. microplus* species of the clade A and to *Amblyomma mixtum* species.

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The potential role of migratory birds in the spread of ticks and tick-borne pathogens in Baltic region

Algimantas Paulauskas, Jana Radzijevskaja, Vesta Matulaitytė

Email: algimantas.paulauskas@vdu.lt
Department of Biology, Vytautas Magnus University, Kaunas, Lithuania

Abstract/Resumen

The importance of migratory birds in the maintenance and the global dispersal of arthropod-borne pathogens has been increasingly recognized during past decade. Every spring and autumn, myriads of birds migrate over Baltic region from northwest to southeast and from northeast to southwest flyways. The aim of this study was to assess the importance of passerine migratory birds in spreading of tick-borne infectious agents in Baltic region. For this propose we examined tick-borne bacterial pathogens in ticks collected form passerine birds during spring and autumn migration at Ventės ragas ornithological station (Western Lithuania) in 2017-2019. A total of 3349 birds belonging to 34 species were caught and 1793 *Ixodes ricinus* ticks were collected from 528 birds of 15 species. Organs (liver, heard) from 72 dead goldcrest (*Regulus regulus*) specimens were also collected for analysis. For simultaneous detection of DNA from 7 different genera of tick-borne bacteria, collected ticks and tissues samples were analyzed using multiplex PCR followed by automated reverse dot blot hybridization based on DNA-Flow Technology (hybrSpot). Eight tick-borne bacterial pathogens were identified in bird-feeding ticks: four *Borrelia* species (*B. garinii, B.valaisiana, B. afzelii*, and *B. miyamotoi*), two *Rickettsia* species (*R. helvetica* and *R. monacensis*), *Anaplasma phagocytophilum* and *Candidatus* Neoehrlichia mikurensis. *Borrelia* spp. and *R. helvetica* were detected with higher prevalence compared with other pathogens. Infected ticks were found on 10 species of birds: *Erithacus rubecula, Troglodytes troglodytes, Sturnus vulgaris, Turdus merula, Pyrrhula pyrrhula, Fringilla coelebs, Spinus spinus Prunella modularis, Phylloscopus trochilus* and *Phoenicurus phoenicurus.* Our study demonstrates that these species of passerine migratory birds may support the circulation and spread of tick-borne bacterial pathogens of medically importance in Baltic region. *A. phagocytophilum* DNA was detected in 41.6 % of liver and head samples of goldcrests. These findings suggest that goldcrest could act as potential reservoir host for *A. phagocytophilum.*

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P-01 Assessment of bacterial selection strategies for biological control of *Rhipicephalus microplus*

Liszoe Galdós Betartez*, Ramón Franco Rodríguez, Zoila abad Márquez, Néstor Mora González, Laritza Dominguez Rabilero, Idania Cepero Reyes, Raúl González Ríos, Andrés Serradelo Leal.

Email: liszoe.galdos@cigb.edu.cu

Center for Genetic Engineering and Biotechnology, Camagüey, Cuba.

Abstract/Resumen

Rhipicephalus microplus are obligate blood-feeding arthropods that are distributed worldwide and denote a serious hazard to both human health and animal production. *R. microplus* are controlled at present mostly by chemical acaricides. However, biological control is becoming an increasingly attractive approach to tick management. The entomopathogenic fungi are good candidates as biocontrol agents but they have some disadvantages. Some bacteria show pathogenicity to ticks but the specific biology of ticks and the little information available, further research is required to elucidate the mechanism of bacterial pathogenicity. Thus, this study aimed to describe methodologies for obtaining tick antagonist bacteria. We selected several sources of isolation as they were: entomopathogenic nematode, dead ticks, ticks killed by entomopathogenic nematode and dead beetle. We isolated several bacterial strains as possible tick controllers. Batches of larvae ticks were immersed in a suspension of 109 cfu/ml of each strain. All treatment and control groups were observed for 30 days, and the larval mortality was assessed. The effect of the isolates from ticks killed by entomopathogenic nematode tested herein on larvae of *R. microplus* showed a significantly higher mortality than those of the control groups ($p < 0.05$). This study demonstrates that we possess potential strains of interest as a biological agent against ticks.

P-02 *Bartonella* spp. in stray cats and questing ticks from Portugal mainland

Torrejon E[a], Sanches GS[a,b], Couto J[1a], Antunes S[a,b], Domingos A,[ab].

Email: adomingos@ihmt.unl.pt

[a]Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira, 100, 1349-008 Lisboa, Portugal. [b]Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira, 100, 1349-008, Lisboa, Portugal.

Abstract/Resumen

Bartonella spp. comprises small, facultative and fastidious gram-negative intracellular bacteria with worldwide distribution, and are considered neglected zoonotic pathogens, whose transmission to humans occurs through contact with infected mammals and blood-sucking arthropods. Cats are the main reservoir host for at least three *Bartonella* spp.: *B. henselae* and *B. clarridgeiae*, both causative agents for cat scratch disease, and *B. koehlerae* agent for endocarditis, in humans. Although fleas play the major role in transmission of feline *Bartonella*, other potential arthropod vectors have been identified to harbor *Bartonella* DNA such as ticks, thus representing an important group for *Bartonella* surveys. Considering the scarce number of studies addressing the occurrence of *Bartonella* in Portugal, the present study focus on the molecular identification and characterization of *Bartonella* spp. circulating in stray cats and in questing ticks. Tick collections were performed using the drag-flag method from 2012-2018 in the 18 administrative regions of mainland Portugal and blood from non-domiciled cats participating in the sterilization program promoted by Casa dos Animais de Lisboa was screened. Molecular detection and quantification of *Bartonella* DNA were carried out by qPCR targeting a fragment of *nuoG* gene. Positive samples were characterized by a cPCR targeting the *gltA* and *ribC Bartonella* spp. genes. From 124 cat blood samples, 20% were positive for the *Bartonella nuoG*. From those, 15 were characterized as *B. henselae*, 11 as *B. clarridgeiae*, and 1 presented a co-infection of both species. Regarding tick surveillance, from 236 adult ticks sampled (154 *Ixodes ricinus* and 82 *Rhipicephalus sanguineus* sensu lato), all were negative for the *nuoG* gene. Despite our results were not supportive to ticks as competent vectors for *Bartonella*, more studies with new approaches need to be performed in order to clarify the role of ticks as vectors of *Bartonella* spp.

P-03 Cross-amplification of *Rhipicephalus microplus* microsatellite loci in Cuban *Riphicephalus* sanguineus tick strain.

Yuselis García[1], Pedro E. Encinosa[1], Katia Rosales[1], Yamil Bello[1], Alier Fuentes[2], Alina Rodriguez-Mallon[1]

Email: yuselys.garcia@cigb.edu.cu

[1]Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology (CIGB).Ave. 31 e/ 158 y 190, Playa. PO Box 6162, Havana 10600, Cuba. [2] National Laboratory of Parasitology, MINAG, Cuba

Abstract/Resumen

Ticks are considered the second most important transmitters of diseases in humans after mosquitoes. They affect domestic animals, wild animals and pets. Immune control is a promising alternative for its control; however there are no vaccines with protective antigens against *Rhipicephalus sanguineus* infestations in dogs. The search for new vaccine antigens against this ectoparasite requires an experimental tick model that provides relevant biological data. A *R. sanguineus* tick colony has been established at the CIGB in order to guarantee the production of tick specimens under standardized conditions to be used as an experimental model in challenge trials to determine the efficacy of anti-tick vaccine candidates. However, inbreeding is an important negative effect in the laboratory maintenance of tick colonies. Consequently, genetic variability studies should be conducted. Microsatellites have become the markers of choice for high-resolution assessment of genetic variation and population structure studies. The present work aims to evaluate the cross-amplification of microsatellite sequences of *R. sanguineus* using primers from *R. (B.) microplus* reported sequences. *R. microplus* and *R. sanguineus* ticks were obtained from the colonies of the National Laboratory of Parasitology and from the "Bejucal 2010" colony established by CIGB, respectively. Total genomic DNA was extracted using the QIAamp genomic DNA kit. A total of 16 microsatellite loci were tested for cross-amplification in *R. sanguineus* ticks using conventional PCR. Positive amplifications were obtained in 10 loci of them. PCR products for both ticks were sequenced to corroborate the specificity of amplicons. Effective cross-amplification was 62.5%. The selected microsatellite loci can be used to assessment genetic variation of specimens from "Bejucal 2010" tick colony.

P-04 Effect of RNAi and antibodies against P0 ribosomal protein on *Ixodes ricinus* ticks.

Pedro E. Encinosa[1], Jan Perner[2], Helena Frantova[2], Jan Erhart[2], Petr Kopacek[2], Alina Rodríguez-Mallon[1]

Email: pedro.encinosa@cigb.edu.cu

[1]Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology (CIGB), P. O. Box 6162, Havana 10600, Cuba. [2] Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic

Abstract/Resumen

Ixodes ricinus is the most widely distributed tick in Europe and is able of transmitting pathogens that affect humans, pets and wild animals. One of the most important diseases caused by the spirochetes of the genus *Borrelia* sp. is Lyme disease, which has increased in recent decades in Europe. Currently, there is no vaccine capable of controlling infestations of this tick species. Recently, a peptide from the ribosomal P0 protein (pP0) of *Rhipicephalus* spp. ticks chemically conjugated to Keyhole Limpet Hemocyanin (KLH) has shown in controlled studies high efficacy against infestations of *Rhipicephalus sanguineus* and *Rhipicephalus microplus* ticks. The objective of this work was to study the effect of P0 RNAi and antibodies against pP0 on *I. ricinus* ticks. The expression of P0 gene was silenced by microinjection of P0 dsRNA in *I. ricinus* adult females one day before of feeding them on guinea pigs. The specific gene silencing was confirmed by RT-qPCR. A specific knockdown effect on P0 transcripts of 59%, 60% and 74% was demonstrated in intestine, salivary glands and fat body, respectively. The weight of P0 dsRNA microinjected females was significantly reduced with respect to GFP dsRNA microinjected females used as a control group. One day after the final collection, all P0 dsRNA microinjected females were dead. In contrast, all GFP dsRNA microinjected females were able to finish the oviposition and eggs hatched normally. An immunization experiment was also performed using a chemical conjugate of pP0 with Bm86 in rabbits which were challenged with *I. ricinus* adult ticks. The pP0-Bm86 immunized rabbit reached high specific antibody titers against both antigens. Females fed on pP0-Bm86 vaccinated animal had a statistically significant reduction in their weight when compared with that of females fed on PBS injected animal.

Acknowledgement: Mobility for these experiments was supported by CYTED Network INCOGARR (118RT0541)

P-05 Effective management of waste generated in the IFA production process of the Gavac® vaccine, in the environment of the CIGB Camagüey environmental policy

Odisa Esquivel Avalos, Diasmarys Salinas Rodríguez, Rutdali Segura Silva, Liyoesmin Salinas Rojas, Irian Mendoza Rodríguez, Laura Gabriela Gómez Porto

E-mail: odisa.esquivel@cigb.edu.cu

Grupo Gestión de la Calidad y Asuntos Regulatorios, Center for Genetic Engineering and Biotechnology, Camagüey, Cuba

Abstract/Resumen

The achievement of balance between the environment, society and the economy is essential to meet the needs of the present without putting the future at risk. The biotechnology industry uses raw materials, energy, capital and human labor to generate socially desirable goods, but also, its production processes throw undesirable by-products into the environment, the volume and characteristics of this by-product become a management challenge for the company and of environmental policy. The requirements of the GMP include the protection of people and the environment, which creates the need to design waste management policies. To address this need, three key aspects considered for the management in the wastes generated from the IFA production process of the Gavac® vaccine, recombinant immunogen against ticks *Boophilus microplus*, *Rhipicephalus annulatus* and *Rhipicephalus decoloratus* action. The developed methodology included the design of a master plan for waste management, based on a specific checklist for each stage of the process. The analysis of the different situations led to the design of procedures for waste management, complying with regulatory requirements of GMP and the legislation in force in the country. The disposal and destruction of waste is as another step in the production process, including the appropriate methods to carry it out, analytical checks to demonstrate that the treatment was effective and the training of personnel involved in waste treatment at the point where they generate. The management of the waste generated in the production process was effective and in accordance with the environmental policy of the center and the country, taking into account the emissions to the environment.

Keywords: Gavac®, waste management, GMP, environment, vaccine

P-06 Effects of anti-tick antibodies microinjected in *Rhipicephalus sanguineus* ticks

Cano-Argüelles A.L.[1], Antunes S.[2], Encinosa Guzmán P.E.[1], Cabrera Herrera G.[3], Sanchez G.S.[2], Couto J.[2], Bello Soto Y[1], Domingos A.[2] and Rodriguez-Mallon A.[1]

Email: ana.cano@cigb.edu.cu

[1] Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology, Cuba. [2] Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal. [3] Proteomic Department, Center for Genetic Engineering and Biotechnology, Cuba

Ticks are ectoparasites that can transmit a wide variety of infectious agents that cause diseases in humans and animals. One of the promising methods of controlling tick infestations is vaccination. The immunization with Bm86 antigen has proven largely to be effective in field studies against *Rhipicephalus microplus* ticks. Recently, a peptide from the P0 acidic ribosomal protein of *Rhipicephalus* spp. ticks chemically conjugated to Keyhole Limpet Hemocyanin (KLH) has been assayed as vaccine candidate against different tick species with efficacies around 90%. However, both the biological function of Bm86 and the function of P0 blocked by antibodies and in which localization remain unclear. Few studies have introduced host antibodies in ticks by direct injection into the hemocoel to evaluate the effect of antibodies against different tick proteins. Here we attempted to evaluate the effect of anti-P0 and anti-Bm86 antibodies injected through the anus to *R. sanguineus* female adult ticks. Specific IgGs against P0 and Bm86 were purified from sera of immunized rabbits by affinity chromatography. A group injected with anti-His antibodies was used as negative control group. After 24 h, microinjected ticks were allowed to feed in rabbits with the same quantity of male ticks. The results showed a lower attachment rate in the groups injected with anti-tick antibodies (22% in the group with anti-P0 antibodies, 28.6% with anti-Bm86 antibodies and 86.7% with anti-His antibodies), lower engorgement rate (13.3%, 12.5% vs. 60%, respectively) and a slightly high mortality (45%, 35.7% vs 26.7%, respectively) than the control group. Our current findings suggest the possibility of using anti-tick antibodies injection as a method to study the effect of antigens for the future development of vaccines against these ectoparasites.

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P-07 Efficacy of Neem Oil (*Azadirachta indica*) and Orange Oil against the cattle tick *Rhipicephalus* (*B.*) *microplus* (Acari:Ixodidae) in field condition

Francinea Souza [1,2]; Julia Lampa [1]; Sthephanie Zanelatto [1]; Bianca Silva [2]; Saulo Weber [1]; Gervasio Bechara [1*]

[1] School of Life Sciences, Pontifical Catholic University of Paraná (PUCPR), Curitiba, Brazil; [2] Dexter Latina Indústria e Comércio de Produtos Químicos, São José dos Pinhais, Brazil; [*] gervasio.bechara@pucpr.br

*Rhipicephalus (*Boophilus) *microplus* is the main ectoparasite responsible for economical losses in cattle of (sub)tropical countries due to reduction in weight gain and milk production, hide damage and haemoparasites transmission. Its control has been done with chemical products despite the spread of tick population resistance to several molecules, accumulation of chemical residues in animal products, environmental contamination and risk of non-target organisms. Therefore, more ecological friendly bothanical products has been studied in commercial presentations as alternative to chemical acaricides. The aim of this study was to investigate the efficacy of neem (*Azadirachtina indica*) and orange oils against the cattle tick *R. microplus* in field conditions. The larval packet test (LPT) was used to determine the lethal concentrations for cypermethrin, neem and orange oils being the results expressed as percentages of live and dead larvae in relation to the total number of individuals. After this, commercial formulations of 18 mg neem active ingredient (a.i.) and 12 mg orange a.i. in emulsifiable concentration (EC) form were prepared for spray applications in cattle. Statistics parameters inter molecules as variation coefficient and standard error of estimate were respectivelly 0.989; 0.986 and 5.739; 6.129 for neem and orange oils. The maximum mortality was 100% and dose–response curves were compared between neem and orange oils and a non treated control group. The number of ticks attached to the animal's body in 1, 5 and 7 days after treatment was the main variable analyzed. Drop time from knock down 50% (KD50) curve was determined also. There was significant difference between the control and treated groups being 8.38 hours for neem oil and 9.36 hours for orange oil. It is concluded that neem and orange oils could be formulated in EC form and used to control the cattle tick *R. microplus* as a management method to avoid possible tick resistance to chemical acaricides.

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P-08 *Ehrlichia canis* in *Rhipicephalus* sp. and *Ixodes* sp. ticks in Portugal mainland

Gustavo S. Sanches[a,b], Joana Ferrolhoa,[b], Joana Coutoa,[b], Estefania Navarro[a], Leonardo Moreira[a], Ana Sofia Santos[c], Margarida Santos-Silva[c], Sandra Antunes[a,b], Ana Domingos[a,b*]

[a] Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira, 100, 1349-008 Lisboa, Portugal. [b] Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (GHTM-IHMT-UNL), Rua da Junqueira, 100, 1349-008 Lisboa, Portugal. [c] Instituto Nacional de Saúde Doutor Ricardo Jorge, Centro de Estudos de Vectores e Doenças Infecciosas Dr. Francisco Cambournac (CEVDI), Av. da Liberdade, 5, 2965-575 Águas de Moura, Portugal. *Corresponding author: Ana Domingos, E-mail: adomingos@ihmt.unl.pt , Phone: +351 213652600, Fax: +351 213652105

Abstract/Resumen

Ehrlichia canis is the etiological agent of canine monocytic ehrlichiosis (CME), a multisystemic infectious diseases with veterinary importance. This gram-negative bacterium invades and multiply inside the monocytes and macrophages, causing nonspecific clinical manifestations as fever, anemia, and eventually death. The main biological vector of CME is the *Rhipicephalus sanguineus* sensu lato (s.l.) tick. This group includes two lineages having different geographic distribution, biology, genetics and behavior regarding vector capacity to *Ehrlichia* sp.. The "tropical lineage" is commonly found in tropical areas (America and Sub-Saharan Africa) and is reported as competent vector of *E. canis*, while the "temperate lineage" frequently found in temperate and colder areas (South America, North America and western Europe) is not. Curiously, in Portugal, where the temperate lineage is the only representative of *R. sanguineus* s.l., cases of CME are common. Such contradictory findings suggest the need for further studies to evaluate the role of the temperate lineage (or other tick species) in *E. canis* transmission. A *E. canis* vector candidate is *Ixodes ricinus* due to distribution across Europe and wide range of pathogen transmission.

Therefore, the aim of this study was to evaluate the natural infection of *E. canis* in the temperate lineage of *R. sanguineus* and *I. ricinus* questing ticks collected in Portugal. For this, tick collections were performed from 2012 to 2018 in mainland Portugal. Ticks were collected by dragging-flagging vegetation and morphologically identified under a stereo-microscope. Only ticks identified as *R. sanguineus* s.l. and *I. ricinus* were included in this study. DNA from whole ticks was extracted by using alkaline hydrolysis and its concentration and integrity evaluated. Nested PCR assays were performed targeting the *E. canis* 16SrRNA gene and PCR products sequenced. Preliminary results reveal the presence of *E. canis* in both *R. sanguineus* s.l. and *I. ricinus*

P-09 Evaluation of the antigen rBm86 from a Mexican strain of *R. (B.) microplus* in dogs infested with *R. sanguineus* adult ticks from Mexicali, Mexico

Alma Rossana Tamayo Sosa[1], Yerania Margarita Robles Graham[2], Luis Tinoco Gracia[3], Sergio Arturo Cueto González[4], Alberto Barreras Serrano[5], Víctor Manuel del Villar Perez[6].

Email: almatamayo@uabc.edu.mx

[1] Laboratorio de Inmunología Comparada. Universidad Autónoma de Baja California. Instituto de Investigaciones en Ciencias Veterinarias. Mexicali, Baja California, México. [2] Programa de Maestría en Ciencias Veterinarias. UABC-IICV. Mexicali, Baja California, México. [3] Laboratorio de Salud Pública. UABC-IICV. Mexicali, Baja California, México. [4] Laboratorio de Parasitología. UABC-IICV. Mexicali, Baja California, México. [5] Mejoramiento Genético y Estadística. UABC-IICV. Mexicali, Baja California, México. [6] Laboratorio de Microbiología. UABC-IICV. Mexicali, Baja California, México.

Abstract/Resumen

The Bm86 antigen is a glycoprotein isolated from intestinal cells of *Rhipicephalus (Boophilus) microplus* present in all developmental stages of these ticks. The recombinant form of this antigen has been used to develop commercial vaccines that have shown to be effective against *B. microplus* (TickGARD™ Australian and Gavac™ Cuba) in an integrated control program gradually reducing the use of acaricides, and also has shown efficacy against all stages of *R. sanguineus* due to phylogenetic relation among the Bm86 genes of the *Rhipicephalus* genera. In the present study the effect of rBm86 from a Mexican strain of *R. (B). microplus* was evaluated. Eight female and male dogs were divided into two groups of four: one vaccinated and a non-vaccinated. Vaccinated dogs received two doses of 60µg of rBm86 Mexican strain at one month interval, while the non-vaccinated was given saline solution. All dogs were challenged one month after the last vaccination with 28 adults ticks (14 females and 14 males) placed inside feeding chambers. Engorged ticks were recovered and Collection rate, egg mass and hatchability were determined. Sera collected at 0, 30 and 60 days after vaccination was used for detection of specific antibodies by ELISA. Collection rates of adult females fed on vaccinated and non-vaccinated dogs were not significantly different ($P>0.05$). Neither differences were observed ($P>0.05$) in neither egg mass nor hatch rate of ticks fed from vaccinated and non-vaccinated dogs. Optical densities in ELISA test did not showed a significant difference ($P>0.05$) between vaccinated and non-vaccinated dogs at any time. We concluded that rBm86 Mexican strain did not reduce viability and biotic potential of the *R. sanguineus* in Mexicali, and may be due to genetic diversity between the Bm86 proteins from the Mexican strain of *R. (B). microplus* and the Rs86 of *R. sanguineus* from this area.

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P-10 Evaluation of the ozonized sunflower oil effect on *Rhipicephalus sanguineus* ticks

Pedro E. Encinosa Guzmán[1], Yamil Bello Soto[1], Zullyt Zamora[2], Oscar Ledea[2], Marisdania Joglar Piñeiro[1], Mario Pablo Estrada[1] and Alina Rodríguez-Mallon[1].

Email: yamil.bello@cigb.edu.cu

[1]Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology (CIGB), P. O. Box 6162, Havana 10600, Cuba. [2] Centro Nacional de Investigaciones Científicas (CNIC), Cuba

Abstract/Resumen

Ozone (O_3) is a gas composed of three oxygen atoms. Although it is not a radical molecule, it is far more reactive than oxygen and a strong oxidative agent which has the ability to oxide plasma membrane of all microorganisms including bacteria, virus and fungus, and eventually shred these microorganisms. Therefore, ozone has been used as a disinfectant for many years. As it is an unstable gas that cannot be stored; it is practical and cost-effective to use O_3 in combination with vegetable oils. Ozonized oils contain the stabilized O_3 molecule, as an ozonide between the double bonds of a monounsaturated fatty acid such as oleic acid, which is ideal for topical use of O_3. The aim of the current study was to assess the efficacy of ozonized sunflower oil on *Rhipicephalus sanguineus* ticks. The larvae immersion test was performed using three different concentrations of ozone in sunflower oil. A concentration dependent effect on larvae viability was obtained. One hundred percentage of larvae mortality was obtained with the highest concentration of ozonized oil and more than 95% with the middle concentration. Both mortalities were significatively more higher than larvae mortalities obtained in the control group and in the group treated with the lowest concentration of ozonized oil.

P-11 Gavac®: Renewal of the Sanitary Registry in Cuba

Salinas, D [1], Mena, J[2]; Segura, R[1]; Moreira, A[1]; Montoya, N[1]; Gonzalez, N[1]; Basulto, R[1]; Mendoza, I[1]; Pena, W[1]

[1] Center for Genetic Engineering and Biotechnology, Camagüey, Cuba
[2] Center for Genetic Engineering and Biotechnology, Havana, Cuba
E-mail: diasmarys.salina@cigb.edu.cu

Abstract/Resumen

Gavac®; immunogen against ticks *Rhipiicephalus (Boophilus) microplus*, *R. annulatus* and *R. decoloratus*; is successfully applied in Cuba as part of integrated pest control in cattle since the 90s of the last century. In 1998, it registered as an injectable for veterinary use in Cuba. For the permanence of Gavac® in the national market, it is necessary to renew its sanitary registry, so the objective of this work was to prepare and achieve the approval of the new edition of the document that will allow the continuity of the production, application and commercialization of this product in Cuba. To make the file, the approved edition of 2013 was used as a base and the procedures and records were consulted, change records, validation, stability, among others and the regulations applicable to biological products for veterinary use established by VICH and CAMEVET. The prepared file contains eighteen improvements introduced in the manufacturing and control, nine inclusions and seven updates. Because of inspections and review of the delivered document, the regulatory authority, the Office of Registration of Veterinary Products attached to the Institute of Veterinary Medicine; granted the renewal of the sanitary registration for the product and the sanitary licenses of manufacture and commercialization. The quality of the current edition of the Sanitary Registry Dossier and the results obtained in the on-site inspections determined the renewal of the same that guarantee the current permanence of Gavac® in the national market.

Keywords: immunogen, renewal, sanitary registration.

P-12 Non-conformity Management System in the Gavac® Immunogen production of CIGB-Camagüey

Mendoza, I[1], Salinas, D , Segura, R , Pérez, A , Salinas.

[1] Quality Department, Center for Genetic Engineering and Biotechnology, Camagüey, Cuba

E-mail: irian.mendoza@cigb.edu.cu

Abstract/Resumen

The design and implementation of a quality management system for nonconformities generated during the manufacturing and distribution in the Gavac® Immunogen production of the Center for Genetic Engineering and Biotechnology of Camagüey (CIGB) is focused on the satisfaction of needs and expectations of all interested parties, both internal and external. The objective of this work is to analyze the process of management of nonconformities from the notification stage to the evaluation, determining the efficiency and effectiveness indicators of the system. The evaluated years were 2017 and 2018. The information was processed through Microsoft Excel. In the first year studied 393 nonconformities were notified and in the second 130; of them evaluated as criticism 28 and 10, respectively. A decrease in the number of non-conformities detected was detected due to greater compliance with Good Practices and rigorous internal inspections. The process of corrective and preventive actions within the quality management in the institution makes it possible to guarantee greater availability and quality of the product, according to the established quality specifications.

Keywords: Biopharmaceutical products, quality management system, non-conformance system.

P-13 PHYTOCHEMICAL PROFILE AND INSECTICIDE POWER FROM THE LEAVES AND BARK EXTRACT OF THE NEEM TREE

Silvia Denise Peña Betancourt.
Laboratory of Toxicology. Universidad Autónoma Metropolitana- Xochimilco, Coyoacan Mexico. Email spena@correo.xoc.uam.mx

Abstract/Resumen

The ectoparasites problem in pets has been a problem for many decades. Synthetic drugs are commonly used, however they present several inconvenients. *Azadirachta indica* (Neem) tree, grows in tropical weather and its oil has been tested for its insecticidal effect. The aim of this study was to identify and compare the phytochemical profile of the alcoholic extracts from the leaves and bark of the Neem tree and also to evaluate its insecticidal effect in dogs. The plant was collected in the State of Morelos, Mexico, during winter 2018. It was determined the presence of alkaloids, tannins, saponins and triterpenoids by qualitative techniques. The biological test was conducted in six dogs, which were administered 7 mL of the alcoholic extract from leaves and bark; a Neem commercial insecticide and a commercial chemical insecticide, as positive controls. The results showed a higher yield of the bark extract than the one in leaves 20% vs 9.4%; with a higher content of saponins (0.25% vs. 0.01%), while in the leaves extract, a higher content of tannins (5.43 mg mL vs. 1.75 mg mL). It is concluded the insecticide effectiveness in alcoholic extracts from leaves and bark of Neem. The bark extract was even higher compared to the leaves extract and commercial insecticide.

Keywords: *Azadirachta indica*, Neem, insecticide, dogs

P-14 Quality management in the production of Gavac® in 2018

Salinas, Liyoesmin*; Mendoza, Irian; Segura, Rutdali; Salinas, Diasmarys

Grupo de Aseguramiento de la Calidad y Asuntos Regulatorios, Centro de Ingeniería Genética y Biotecnología, Camagüey, Cuba. Email: liyoesmin.salinas@cigb.edu.cu

Abstract/Resumen

Gavac® is a recombinant immunogen against ticks *B. microplus*, *Rannulatus* and *R. decoloratus*. Quality management in the production of vaccines ensures that manufacturing is carried out according to the codes of good practice and that the product is pure, safe and effective. The objective of this investigation is to analyze the process of quality management in Gavac® productions in 2018. The aspects to be considered were the documentation of the process, release of lots, detected nonconformities and inspections. The Microsoft Excel spreadsheet was used to process the data. During 2018, 47 new editions of documents related to this production process were carried out: 20 operating procedures, 15 production master records, 6 general records, 4 quality specifications and 2 inspection and test plans. Eight batches of Active Pharmaceutical Ingredient (API) were released, of immunogen 19 lots were produced equivalent to 3 459 750 doses; of them 15 were released and are in finished production, the remaining 4 constitute production in process. Of the 16 notified non-conformities, only 3 were critical. The quality management in the production process of Gavac® made it possible to ensure the availability of a uniform product and in accordance with the quality specification.

Keywords: biofarmaceutic industry, Gavac®, quality management

P-15 Rapid evaluation of anti-Bm86 antibody titers by the lateral flow immunochromatographic system HeberFast Line Gavac.

Milagros Vargas[1], Yeni Hernández[2], Viviana Pluma[2], Enrique Perez[3], Daymi Abreu[3], Carlos Montero[1], Ayme Oliva[1], Elaine Santana[1], Danny Perez[1], Alianne Fundora[1], Carlos A. Duarte[4], Marisela Suarez[1].

[1] Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology (CIGB).Ave. 31 e/ 158 y 190, Playa. PO Box 6162, Havana 10600, Cuba; [2] Unit of Central Laboratories of Agricultural Health; [3] Center for Genetic Engineering and Biotechnology, Sancti Spiritus, Cuba; [4] Pharmaceuticals Department, CIGB, Havana, Cuba.

Email: milagros.vargas@cigb.edu.cu.

Abstract/Resumen

Rhipicephalus (Boophilus) microplus cattle tick is a scourge to livestock production in our country. Gavac immunogen is used within an Integrated Control Program to control this ectoparasite. The mechanism of action of Gavac is based on the induction of antibodies against the Bm86 antigen of the cattle tick gut; therefore the development of fast methods to monitor those antibody titers in the field is of upmost importance. A lateral flow immunochromatographic system (HeberFast® Line Gavac) was developed as a fast method to detect anti-Bm86 titers in the serum. To evaluate the performance of this method, 598 sera from immunized animals were evaluated. These samples were assigned into 3 groups (high, medium and low) according to its anti-Bm86 titers previously measured by Bm86 ELISA. Another 100 samples from non-immunized animals were included. The sensitivity, specificity, concordance with the Bm86 ELISA reference system, batch to batch consistency, and differences between analysts were calculated. The system showed sensitivity values of 81.6%, 82.2% and 81% for the three batches, respectively; a specificity of 96.7%, 94.6% and 93.3%, and good agreement (75%, 74% and 71%) with respect to Bm86 ELISA. The overall effectiveness of the diagnosis was 87.6%, 87.1% and 85.9%). Similarly, the method showed good batch to batch consistency, and its effectiveness was independent of the analysts. The results of this study indicate that HeberFast® Line Gavac is a useful tool for the serological surveillance of the Integrated Control Program against cattle tick.

Key words: lateral flow immunoassay, Bm86, rapid diagnosis, antibodies.

P-16 *Rhipicephalus microplus* resistance to acaricides in Brazil: partial results.

Zelina Dos Santos Freire[1], Juliane Francielle Tutija[1], Guilherme Henrique Reckziegel[1], Milena Batista Machado Araripe[1], Tom Strydom[2], Daniel Rodrigues[2], Fernando de Almeida Borges[1]

Email: drodri12@gmail.com

[1] School of Veterinary Medicine and Animal Science, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil; [2] MSD Animal Health

Abstract/Resumen

The severe Brazilian scenario of resistance of *Rhipicephalus microplus* to spray acaricides is widely described in the literature. However, so far there are few studies with pour on or injectable acaricides, which results in a lack of current knowledge about drug resistance. The objective of this study was to determine the resistance levels to commercially available acaracides, on randomly selected commercial farms in Brazil. This study was a randomized, open label, resistance study. For this, 42 commercial farms were selected form seven States for tick collection. The adult Immersion Test (AIT) was used to evaluate fluazuron. Alpha-cypermethrin impregnated papers was used as an indicator of resistance against synthetic pyrethroids, chlorfenvinphos as a general indicator of organophosphorus resistance, and amitraz as an indicator for formamidine resistance in the Larval Packet Test (LPT). Moxidectin and fipronil were evaluated by the Larval Immersion Test (LIT). In order to perform paired tests, the Porto Alegre strain (POA), characterized as susceptible to all acaricides, was used. To date, we have the results of the tests performed on 36 farms. Some chemical groups are in critical resistance status. All evaluated samples were resistant to alpha-cypermethrin, demonstrating the severe resistance status to synthetic pyrethroids in Brazil. The second active ingredient with the largest number of resistant samples was amitraz, with 87.1% of the farms. 60 and 73.3% of the samples were classified as resistant to fluazuron and fipronil, respectively. However, only samples from five farms were evaluated for fluazuron, because females had already started laying eggs before arriving at the laboratory or due to reduced number of viable ticks. In 64% of the evaluated farms, moxidectin was still effective and chlorfenvinphos in 74.3%. These results demonstrate that there is resistance to all active ingredients available in the Brazilian market for the control of *R. microplus*.

P-17 Silk Fibroin Nanoparticles as a new carrier of a tick peptide (pP0): a novel strategy for the development of anti-tick vaccines

Lozano-Pérez, AA[1]; Pagán, A[1]; Aznar-Cervantes SD[1]; Encinosa-Guzmán, PE[2]; Bello Y[2]; Cano-Argüelles, AL[1]; Cenis, JL[1] and Rodríguez-Mallon, A.[2]

Email: anapagan@um.es

[1]Department of Biotechnology, Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA)., Murcia, Spain; [2]Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology (CIGB).Ave. 31 e/ 158 y 190, Playa. PO Box 6162, Havana 10600, Cuba.

Abstract/Resumen

Silk Fibroin Nanoparticles (SFNs), obtained from the silk fibroin produced by the silkworm *Bombix mori L*., have been showed as promising carriers for different biomolecules due to their unique features in terms of morphology, size, wide range of available chemical groups on their surface and biodegradability. This nanoparticles not only show a narrow distribution of hydrodynamic sizes, ranging from 120 to 160 nm in diameter, but also with a high proportion of b-sheet conformation of the silk proteins, what confer them a high stability in aqueous media.

Recently, a 20 amino acid peptide from the acidic ribosomal protein P0 of *Rhipicephalus* sp. ticks has been used successfully as vaccine candidate against *R. sanguineus*, *R. microplus* and *Amblyomma mixtum* ticks when conjugated to keyhole limpet hemocyanin (KLH). These results, together with the conserved sequence of the P0 peptide among ticks, suggest that this antigen could be a vaccine candidate with an interesting broad spectrum. However, selecting the right strategy to efficiently generate large quantities of antigen with suitable vaccine properties is a considerable challenge.

Here we present an example of silk nanoparticles acting as new carrier for the peptide pP0, which is linked to the surface of the SFNs through the well-known carbodiimide-assisted and maleimide coupling chemistry in order to increase the stability of the peptide in the harsh environment of the tissues and blood. Thus we offer the SFNs as a versatile platform with several possibilities of coupling molecules in the surface with multiples applications in nanomedicine.

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P-18 Strategy against the fly *Haematobia irritans* and the tick *Rhipicephalus microplus* with the use of Effipro Bovis in a bovine herd

Alier Fuentes-Castillo, Yuniel Hernández-Rodríguez , Dalia Quintana-Torrente, Rafmary Rodríguez-Fernández, Luis Méndez-Mellor.

Email: alierfuentesc1315@gmail.com
Laboratorio Nacional de Parasitología. Autopista San Antonio de los Baños, km 1½, San Antonio de los Baños, Artemisa, Cuba. CP 38100.

Abstract/Resumen

The control of parasite populations affecting cattle herds is an urgent need in tropical areas, where the presence of ectoparasites is a serious threat to animal health. The tick *Rhipicephalus microplus* as well as the fly *Haematobia irritans*, cause considerable economic damages, so the control of ectoparasites in animals has been very important, demanding the use of chemical agents for an effective and safe control either for animals or for the environment. The objective of this study was to determine the effectiveness of Fipronil (Effipro Bovis) in the control of *R. microplus* and *H. irritans* in cattle under field conditions, applying field rotation as a strategy. The study was carried out in a herd of 14 animals: Holstein and mestizo Holstein F1 naturally infested. Animals were divided into the control and treated group; all of them evaluated before and after treatment. Three consecutive trials were conducted, applying the treatment with Effipro Bovis, each time infestation levels were reached above 10 ticks per animal. As a result of the first trial, there was a reduction in infestation intensity to 0.9 ticks per animal (97.6 %) and, for *H. irritans*, 0.8 flies per animal (99.1 %). A high control percentage was observed in the populations of ticks *R. microplus* in the first 24 hours, and flies *H. irritans* in the days following the treatment. The product Effipro Bovis kept controlled *R. microplus* populations for 35 days and *H. irritans* for 84 days, with an intermediate treatment of the product 42 days after the application.

P-19 Thermal stability studies over active pharmaceutical ingredient of Gavac® inmunogen

R. Segura[1], A. Alfaro[1], A. Moreira[1], L. Gómez[1], D. Salinas[1], E. Pérez[1], I. Zaldívar[1], W. Pena[1], A. Olazábal[1], I. Sánchez[1], R. Basulto[1], I. Mendoza[1], N. Montoya[1], A. Pérez[2]

[1]Centro de Ingeniería Genética y Biotecnología (CIGB) de Camagüey, Circunvalación Norte y Avenida Finlay. e-mail: ruthdaly.segura@correodecuba.cu
[2]Facultad de Ciencias Aplicadas a la Industria, Universidad de Camagüey, Carretera Circunvalación Norte, Km. 5½, e/ Camino Viejo de Nuevitas y Ave. Ignacio Agramonte, Camagüey, Cuba. CP 74650.

Abstract/Resumen

At present, the thermal stability studies are fundamental stages in the development of biotechnological and pharmaceutical processes, since they determine the optimum conditions to be taken into account during the handling and storage of the different products obtained. In the present work two thermal stability studies on the Active Pharmaceutical Ingredient (IFA) of the Gavac® inmunogen are carried out, related to: 1) Increasing the duration of the IFA up to a period of 30 days at a temperature of 2 - 8 ºC, and 2) Establish the number of times the IFA can be frozen and thawed for a period of 90 days at a temperature of - 20 ºC, all without affecting the quality parameters established by the Quality Control System Of the Center for Genetic Engineering and Biotechnology (CIGB) of Camagüey. According to the results obtained it can be concluded that the IFA is stable for a period of 30 days at a temperature of 2 - 8 ° C, and that can be subjected to 5 freezes and thaws for a period of time of 90 days a - 20 Without adversely affecting its main quality parameters.

Keywords: Active Pharmaceutical Ingredient, Quality control, Thermal stability.