



CUBA - 2024

11TH TTP
CONFERENCE



Cuba

<https://www.zooparaz.net/ttp11>

SCIENTIFIC PROGRAM ABSTRACT BOOK

TTP.11

11th Tick & Tick-borne
Pathogen Conference

Cuba 2024

TTP Conference is one of the most important conventions in the world devoted to taxonomy and evolution of ticks and tick – borne pathogens, their ecology and epidemiology, pathogenesis, diagnosis and strategies for their control including immunity and vaccines among others.

✉ alina.rodriguez@cigb.edu.cu

Organized by

CIGB CENTRO
DE INGENIERÍA GENÉTICA
Y BIOTECNOLOGÍA



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1st - 6th

September, 2024

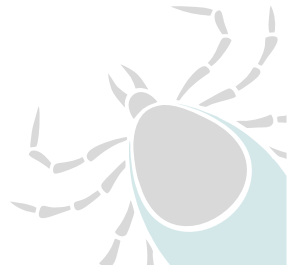
Havana, Varadero, Cuba



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CONTENT

Scientific committee	3
Organizing committee	4
Sponsors	5
Main topics	6
General program	7
Scientific program	8
Plenary lectures	31
Oral presentations	37
Posters	147
List of deleates	251



INTERNATIONAL SCIENTIFIC COMMITTEE



PhD. Ala Tabor



PhD. Alejandro Cabezas



PhD. Ana Gonçalves



PhD. Andrei Mihalca



PhD. Consuelo Almazán



PhD. Domenico Otranto



PhD. Filipe Dantas



PhD. Ladislav Šimo



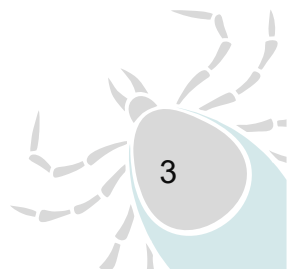
PhD. Marcos Rogério



Prof. Rosangela Zacarias



PhD. Sandra Antunes



ORGANIZING COMMITTEE



PhD. Mario Pablo Estrada García
Director of Agricultural Researches
Center for Genetic Engineering and Biotechnology (CIGB)



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Head of Animal Biotechnology Department
Center for Genetic Engineering and Biotechnology (CIGB)



PhD. Yamila Carpio Gonzáles
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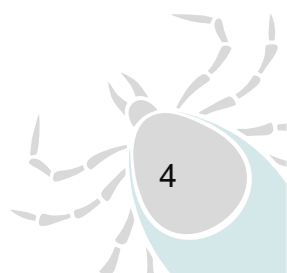
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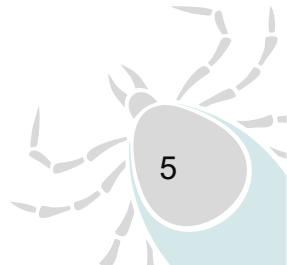




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SPONSORS



MAIN TOPICS



Pathogenesis of ticks and tick-borne pathogens



Ecology and epidemiology of Ticks and tick-borne Pathogens



Tick microbiome and genomics



Diagnosis and strategies for control of ticks and tick-borne pathogens including immunity and vaccines



Special Symposium • Tick microbiota”
Organized by PhD. Alejandro Cabezas



Special Symposium • Tick Physiology and Metabolism”
Organized by PhD. Ladislav Simo



Special Symposium • Tick-borne diseases of dogs and cats”
Organized by Professor. Gad Baneth



Special Symposium • Taxonomy and evolution of ticks and tick-borne pathogens”
Organized by PhD. Filipe Dantas Torres



Special Symposium • Livestock anti-tick vaccines and commercialization challenges”
Organized by PhD. Ala Tabor



Special Symposium • Tick-borne diseases of bovines”
Organized by PhD. Consuelo Almazán & PhD. Juan Mosqueda

GENERAL PROGRAM

Sunday September 1 st	Monday September 2 nd	Tuesday September 3 rd	Wednesday September 4 th	Thursday September 5 th	Friday September 6 th
<p>9:00-17:00 TTP11 Registration Melía Cohiba</p> <p>17:00-17:30 Opening Ceremony (Room Cetro, Melía Cohiba)</p> <p>17:30-18:30 Plenary about CIGB: PhD. Mario P. Estrada</p> <p>18:30-19:30 Plenary lecture of honored invited speaker: Prof. Pat Nuttall</p> <p>20:00 Welcome Dinner and party (Habana Café, Melía Cohiba)</p>	<p>8:30-9:30 Plenary Lecture of honored invited speaker: Prof. Petr Kopacek (Room Cetro, Melía Cohiba)</p> <p>9:30-11:00 Symposium Tick microbiota</p> <p>11:00-11:30 Coffee Break</p> <p>11:30-13:30 Symposium Tick microbiota</p> <p>13:30-15:00 Group photo Lunch</p> <p>15:00-17:00 Symposium Tick microbiota</p> <p>17:00-18:00 Poster Session (Room Cetro, Melía Cohiba)</p> <p>20:00 Free night</p>	<p>8:30-9:30 Plenary Lecture: Dr. Michael Levin (Room Cetro, Melía Cohiba)</p> <p>9:30-11:00 Symposium Tick Physiology and Metabolism</p> <p>11:00-11:30 Coffee Break</p> <p>11:30-13:30 Symposium Tick Physiology and Metabolism</p> <p>13:30-15:00 Lunch</p> <p>15:00-17:00 Symposium Tick Physiology and Metabolism</p> <p>17:00-18:00 Poster Session (Room Cetro, Melía Cohiba)</p> <p>20:00 Cultural activity</p>	<p>8:30-12:00 *Touristic tour Old Havana city</p> <p>City Tour around Modern and Old Havana</p> <p>12:00-14:30 Lunch</p> <p>14:30 Travel to Varadero Resort – Check In</p> <p>20:00 Free night</p>	<p>8:30-9:30 Plenary Lecture: CEO. Lee Fuller, Fuller Laboratories (Room Internacional I, Melía Internacional Varadero)</p> <p>9:30-11:00 Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens</p> <p>11:00-11:30 Coffee Break</p> <p>11:30-13:30 Symposium Pathogenesis of ticks and tick-borne pathogens</p> <p>13:30-15:00 Lunch</p> <p>15:00-17:00 Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens</p> <p>17:00-17:30 Poster awards and Applications for next TTP headquarter (Room Internacional I, Melía Internacional Varadero)</p> <p>21:00-23:00 Beach party</p>	<p>8:30-9:30 Plenary Lecture: PhD. Maxime Madder, Clinglobal (Room Internacional I, Melía Internacional Varadero)</p> <p>9:30-11:00 Symposium Taxonomy and evolution of tick – borne pathogens</p> <p>11:00-11:30 Coffee Break</p> <p>11:30-13:30 Symposium Taxonomy and evolution of tick – borne pathogens</p> <p>13:30-15:00 Lunch</p> <p>15:00-16:00 Closure Ceremony (Room Internacional I, Melía Internacional Varadero)</p> <p>16:00 Return to Havana</p>



SCIENTIFIC PROGRAM

Sunday September 1st / Meliá Cohiba

- **17:30-18:30:** The role of agricultural biotechnology in the One Health Commitment Plenary lecture

By: PhD. Mario Pablo Estrada García – CIGB, Cuba – Director of Agricultural Researches

- **18:30-19:30:** Dynamics of tick feeding and why it matters Plenary lecture

By: Prof. Pat Nuttall-Department of Biology, University of Oxford, UK

Monday September 2nd / Meliá Cohiba

- **8:30-9:30:** Kill the Tick' – an endless gameplay one never gets tired of Plenary lecture

By: Prof. Petr Kopáček- Czech Academy of Sciences, České Budějovice, Czechia

Symposium Tick microbiota Room Cetra, Meliá Cohiba



Chairman

PhD. Alejandro Cabezas, INRAE, France

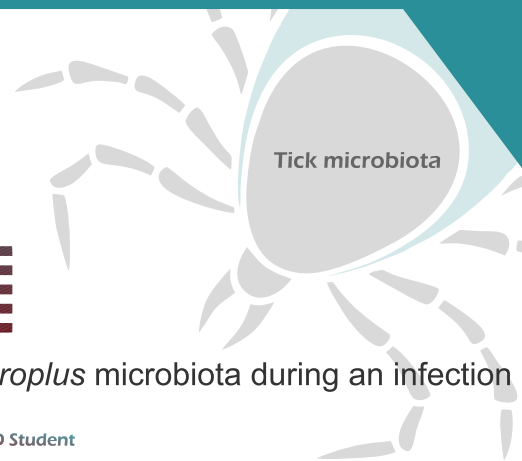
- **9:30-9:45:** Opening session by chairperson
- **9:45-10:30:** Effects of *Rickettsia rickettsii* infection on its vector microbiota
By: Andréa Cristina Fogaça. University of São Paulo, Brazil
- **10:30-11:00:** Dynamic nesting of *Anaplasma marginale* in the microbial communities of *Rhipicephalus microplus*
By: Elianne Piloto Sardiñas. ANSES, INRAE, UMR BIPAR, France and CENSA, Cuba PhD Student
- **11:00-11:30:** Coffee break
- **11:30-12:00:** The potential of anti-microbiota vaccines: a study on the soft tick *Ornithodoros moubata*
By: Ana Laura Cano. IRNASA, Spain PhD Student
- **12:00-12:30:** Host microbiota-induce antibodies alter tick microbiota and block *Borrelia* colonization in ticks
By: Lourdes Mateos Hernandez. ANSES, France



CUBA - 2024

11TH TTP
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Monday September 2nd



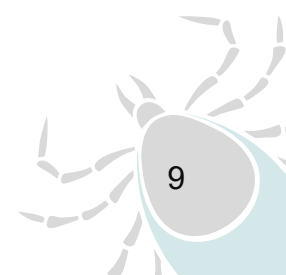
SCIENTIFIC PROGRAM

- **12:30-13:00:** Composition and topology of the *Rhipicephalus microplus* microbiota during an infection with *Babesia bovis*
By: Iván Corona Guerrero. Autonomous University of Querétaro, Mexico **PhD Student**
- **13:00-13:30:** Differential nested patterns of bacterial pathogens and endosymbionts across *Rhipicephalus microplus* ontogeny
By: Lianet Abuin Denis. CIGB, Cuba and ANSES, INRAE, UMR BIPAR, France **PhD Student**
- **13:30-15:00:** Lunch
- **15:00-15:45:** Midichloria bacteria, unique bacterial symbionts of ticks: from evolution to function
By: Davide Sassera. University of Pavia, Italy
- **15:45-16:15:** Exploring innovative strategies for tick-borne pathogen control through multi-antigenic peptide vaccination
By: Apolline Maitre. Ecole Nationale Vétérinaire d'Alfort, France **PhD Student**
- **16:15-17:00:** Defining Tick Microbiota
By: Alejandro Cabezas. INRAE, France

Posters (17:00-18:00)

Posters of all symposia will be presented on Monday September 2nd and Tuesday September 3rd in the room Cetro of the Meliá Cohiba hotel

- **P01:** Trying to disentangle the transmission cycle of *Anaplasma marginale* through *Rhipicephalus microplus* microbiome characterization at the organ scale
By: Marisa Diana Farber. INTA-CONICET, Argentina
- **P02:** Unraveling holobiont-holobiont interactions in host-ectoparasite systems
By: Štefánia Skičková. Pavol Jozef Šafárik University in Košice, Slovakia
- **P03:** Culture-dependent analysis of the microbiome of *Amblyomma aureolatum* and *Amblyomma sculptum*
By: Sylvie Sevcikova. Czech University of Life Sciences Prague, Czech Republic



SCIENTIFIC PROGRAM

Symposium Tick-borne diseases of dogs and cats Room La Corona, Meliá Cohiba



Chairman

Prof. Gad Baneth, The Hebrew University, Israel

- **9:30-10:00:** When bacteria and ticks work together – how dogs deal with Lyme borreliosis
By: Reinhard. Faculty of Veterinary Medicine, Munich, Germany
- **10:00-10:30:** Transmission of canine filarial worms by ticks
By: Domenico Otranto. Università degli Studi di Bari, Italy
- **10:30-11:00:** Canine monocytic ehrlichiosis – an update
By: Shimon Harrus. Hebrew University of Jerusalem, Israel
- **11:00-11:30:** Coffee break
- **11:30-12:00:** Ticks and tick-borne pathogens of carnivores in Europe: the wild-domestic interface
By: Andrei Mihalca. USAMV Cluj-Napoca, Romania
- **12:00-12:30:** Pathogenesis and treatment of canine babesiosis
By: Gad Baneth. The Hebrew University, Israel
- **12:30-13:00:** Tick-borne diseases in dogs and cats in Latin America
By: Filipe Dantas-Torres. Aggeu Magalhães Institute, Brazil
- **13:00-13:30:** Tick-borne diseases of dogs and cats in the Indian sub-continent
By: Sangaran Arumugam. Tamilnadu Veterinary and Animal Sciences University, Chennai, India
- **13:30-15:00:** Lunch
- **15:00-15:30:** Anaplasmosis of dogs and cats
By: Smaragda Sotiraki. Veterinary Research Institute ELGO-DIMITRA, Greece
- **15:30-16:00:** Canine and feline hepatozoonosis – coevolution of parasites with their hosts
By: Alicia Rojas. University of Costa Rica
- **16:00-16:30:** Broadening the One health perspective: Tick-borne diseases shared by men, pets and their environment
By: Hein Sprong. National Institute of Public Health and Environment (RIVM), The Netherlands
- **16:30-17:00:** The first report of *Hepatozoon felis* in a domestic cat in Moscow (Russia)
By: Ekaterina Radyuk. Central Research Institute of Epidemiology, Russia

SCIENTIFIC PROGRAM

Tuesday September 3rd / Meliá Cohiba

- **8:30-9:30:** What does it cost to be a vector: Effects of tick-borne pathogens on the survival and behavior of ticks

By: Dr. Michael Levin, CDC, Atlanta, USA (Retired)

Plenary lecture

Symposium Tick Physiology and Metabolism Room Cetro, Meliá Cohiba



Chairman

PhD. Ladislav Simo, INRAE, France

- **9:30-10:15:** Water management of *Ixodes ricinus* and *Dermacentor marginatus*, two hard tick species in Central Europe with different life cycle strategies Key talk
By: Olaf Kahl. Tick-radar GmbH, Germany
- **10:15-10:45:** These legs are made for sensing: Carbon dioxide enhances *Ixodes* responsiveness to tactile cues
By: Carola Städele. University of Göttingen Medical Center, Germany
- **10:45-11:00:** Unraveling tick lipid metabolism: 'Omics' and biochemistry perspectives
By: Tereza Kozelková. Institute of Parasitology, BC CAS, Czech Republic
- **11:00-11:30:** Coffee break
- **11:30-12:15:** Functional properties of *Ixodes ricinus* cholinergic receptors expressed in the synganglion Key talk
By: Steeve Thany. Université d'Orléans, France
- **12:15-12:55:** Activities of two types of axonal muscarinic acetylcholine receptors mediate formation of saliva cocktail in the tick *Ixodes ricinus*
By: Ladislav Simo. French National Research Institute for Agriculture, Food and Environment
- **12:55-13:30:** The pleiotropic action of a tick salivary serpin on vertebrate haemostasis and its effect on psoriasis-like skin inflammation
By: Mohamed Amine Jmel. Institute of Parasitology, Biology center, Czech Academy of Sciences, Czech Republic
- **13:30-15:00:** Lunch
- **15:00-15:45:** Proteolytic enzymes associated with tick gut tissue Key talk
By: Daniel Sojka. Biology Centre CAS, Czech Republic
- **15:45-16:10:** Inhibition of 4-Hydroxyphenylpyruvate Dioxygenase (HPPD) leads to melanogenic self-catastrophe in ticks
By: David Hartmann. Institute of Parasitology, Biology Centre, CAS, Czech Republic



CUBA - 2024

11TH TTP
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Tuesday September 3rd

Tick Physiology
and Metabolism

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- **16:10-16:30:** The function of the fat body of *Ixodes ricinus* in tick immunity
By: Veronika Urbanová. Biology Centre CAS, Institute of Parasitology, Czech Republic
- **16:30-16:45:** An inhibitor of apoptosis protein is essential to prevent the death of *Amblyomma sculptum* ticks upon a redox imbalance ^{PhD Student}
By: Marcelly Bastos Nassar. University of São Paulo, Brazil
- **16:45-17:00:** Electromagnetic radiation alters the mRNA levels of neuropeptide genes in the *Ixodes ricinus* synganglion ^{PhD Student}
By: Livia Sofrankova. Pavol Jozef Šafárik University in Kosice, Slovakia

Posters (17:00-18:00)

Posters of all symposia will be presented on Monday September 2nd and Tuesday September 3rd in the room Centro of the Meliá Cohiba hotel

- **P04:** Kinin neuropeptide in the midgut endocrine cells and innervation of salivary gland of *Ixodes ricinus*
By: Lianet Abuin Denis. ANSES, France and CIGB, Cuba ^{PhD Student}
- **P05:** Novel neuropeptides involved in the salivary gland innervation of soft tick *Ornithodoros moubata*
By: Livia Sofrankova. Pavol Jozef Šafárik University in Kosice, Slovakia ^{PhD Student}
- **P06:** Thyropin and cystatin proteins from *Ixodes ricinus* saliva: highly selective inhibition of host cathepsins explained by 3D structures
By: Michael Mares. Institute of Organic Chemistry and Biochemistry, Czech Republic
- **P07:** Differential behavioral responses of ticks to radiofrequency electromagnetic radiation exposure
By: Miroslav Bañas. Pavol Jozef Šafárik University in Kosice, Slovakia ^{PhD Student}
- **P08:** Dynamic ultrastructural changes in the midgut of *Ixodes ricinus* nymphs at different feeding stages
By: Veronika Urbanová. Biology Centre CAS, Institute of Parasitology, Czech Republic
- **P09:** Octopamine and α -2 adrenergic-like octopamine receptor in *Ixodes ricinus* salivary glands
By: Sabine Rakotobe. INRAE, France

SCIENTIFIC PROGRAM

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens Room La Corona, Meliá Cohiba



Chairman

Dr. Michael Levin, CDC, Atlanta, USA (Retired)

- **9:30-10:00:** Finding ticks and pathogens before they find us: DAMA protocol combined with citizen science
By: Gábor Földvári. HUN-REN Centre for Ecological Research, Hungary
- **10:00-10:20:** Zoonotic tick-borne pathogens in Serbia: What we know and what we don't know?
By: Aleksandar Potkonjak. University of Novi Sad, Serbia
- **10:20-10:40:** Adapting Retrotransposon Blood Meal Analysis (Rt-BMA) to Identify Tick Hosts and Assess Tick Feeding Preferences in the United Kingdom
By: Holly Broadhurst. University of Salford, United Kingdom
- **10:40-11:00:** Tick-borne infections in Cuba: weaknesses and strengths for the application of One Health strategy from Human Public Health
By: Islay Rodríguez González. PK, Cuba
- **11:00-11:30:** Coffee break
- **11:30-11:50:** MALDI-TOF MS identification of ticks from North of Italy
By: Maria Sampieri. Istituto Zooprofilattico della Lombardia e dell'Emilia Romagna – IZSLER, Italy
- **11:50-12:10:** Phylogenetic inferences based on distinct molecular markers reveals a novel *Babesia* (*Babesia pantanalensis* nov. sp.) and a *Hepatozoon americanum*-related genotype in crab-eating foxes (*Cerdocyon thous*)
By: Marcos Rogério André. UNESP, Campus de Jaboticabal, Brazil
- **12:10-12:30:** Hard ticks from Iberian wolves (*Canis lupus signatus*) and their associated microorganisms, North of Spain
By: Ana M. Palomar. Hospital Universitario San Pedro-CIBIR (Fundación Rioja Salud), Spain
- **12:30-12:50:** Dermacentor ticks and human rickettsioses: a parallel expansion in Eastern France ?
By: Nathalie Boulanger. University of Strasbourg, France

SCIENTIFIC PROGRAM

- **12:50-13:10:** Ticks around and in Paris: presence, pathogens and risk-maps.
By: Krupa Eva. Shaqra Institut Pasteur Paris, France
- **13:10-13:30:** Ticks' diversity and distribution in Saudi Arabia, with insights from ecological niche modeling approaches
By: Abdullah Daria Alanazi. Shaqra University, Saudi Arabia
- **13:30-15:00:** Lunch
- **15:00-15:20:** The Phenology of Ticks and Tick-borne Pathogens in a University Green Zone in Georgia, USA
By: Marina Eremeeva. Georgia Southern University, USA
- **15:20-15:40:** Ecology and life cycle of *Otobius megnini* (Dugès, 1884) in Central Mexico
By: Consuelo Almazán. Autonomous University of Queretaro, Mexico
- **15:40-16:00:** Clinical, epidemiological and serological findings in Cuban individuals bitten by ticks
By: Rene Díaz Fernández. IPK, Cuba
- **16:00-16:20:** Tick-borne pathogen distribution in tick tissues collected from dromedary camels in Kenya: Predicting vector competence
By: Rua Khogali. International Centre of Insect Physiology and Ecology , Kenya *PhD Student*
- **16:20-16:40:** Which pathogens can the neglected winter ticks (*Haemaphysalis inermis*) carry?
By: Szabó Éva. HUN-REN Centre for Ecological Research, Hungary *PhD Student*
- **16:40-17:00:** A dataset of ticks and tick-borne pathogens of Pakistan raises biosecurity concerns for the country
By: Adil khan. Bacha khan University Charsadda, Pakistan

SCIENTIFIC PROGRAM

Posters (17:00-18:00)

Posters of all symposia will be presented on Monday September 2nd and Tuesday September 3rd in the room Centro of the Meliá Cohiba hotel

- **P10:** Tick-borne pathogens detected in ticks collected from migratory birds in Sardinia, Italy
By: Gaia Muronì. Istituto Zooprofilattico Sperimentale della Sardegna, Italy
- **P11:** Identification and antigenicity of the *Babesia ovis* spherical body protein 4 (SBP4)
By: Sezayi Ozubek. Firat University, Turkey PhD Student
- **P12:** Behavior of the biological cycle of the tick *Rhipicephalus microplus* (Acari: Ixodidae) under laboratory conditions
By: Alier Fuentes. National Parasitology Laboratory, Cuba
- **P13:** Stablishing a national network on the surveillance of tick-borne diseases in Spain. Preliminary results
By: A. Sonia Olmeda. Universidad Complutense de Madrid
- **P14:** Blood pathogens in reptiles from the southeastern part of the USA
By: Barbora Pavláková. Pavol Jozef Šafárik University in Košice, Slovakia PhD Student
- **P15:** Investigation of piroplasmid infection in wild and domestic birds received by fauna enterprises in Rio de Janeiro, Brazil
By: Claudia Bezerra da Silva . Universidade Federal Rural do Rio de Janeiro, Brazil
- **P16:** Molecular characterisation of tick-borne infections in cattle in northern Kenya
By: Dennis Getange. International Centre of Insect Physiology and Ecology (icipe), Kenya PhD Student
- **P17:** *Anaplasma phagocytophilum* as a multi-host pathogen in Slovakia
By: Diana Selyemová. Institute of Zoology Slovak Academy of Sciences, Slovakia
- **P18:** Molecular detection of the hemotropic micoplasm in bats captured in forest fragments of Ponta Grossa, Parana, Brazil
By: Eduarda de Oliveira Silva Lima Machado. Federal Rural University of Rio de Janeiro, Brazil PhD Student
- **P19:** Ticks and tick-borne pathogens in urban and forested areas in the Czech Republic
By: Eva Richtrová. National Institute of Public Health, Czech Republic
- **P20:** Tick infesting wild and domestic animals in northern Italy: Ten years of data collection
By: Giulia Maioli. Istituto zooprofilattico sperimentale della Lombardia e dell'Emilia Romagna, Italy
- **P21:** *Borrelia burgdorferi* s.l. abundance and genetic diversity between urban greenspaces and surrounding hinterland across the UK
By: Grace Plahe. University of Salford, United Kingdom
- **P22:** Grey squirrels *Sciurus carolinensis*: how does an invasive species influence the *Borrelia burgdorferi* ecology in the UK?
By: Katherine August. University of Greenwich, United Kingdom

SCIENTIFIC PROGRAM

- **P23:** Genetic characterization of ticks and tick-borne parasites of selected species of captive and free-ranging felids in South Africa
By: Maphuti Betty Ledwaba. University of South Africa
- **P24:** Patterns of cattle breed sensitivity to the tick *Rhipicephalus microplus*
By: Renato Andreotti. Embrapa, Brazil
- **P25:** Investigating tick-borne pathogens in questing and potential reservoir ticks in Portugal
By: Sandra Isabel Da Conceição Antunes. IHMT, Portugal
- **P26:** *Anaplasma phagocytophilum* in passerines from the Ile-de-France region (France), genetic characterization of variants by groEL, ankA and MLST typing
By: Clotilde Rouxel. Anses, France
- **P27:** Population genomics of *Borrelia burgdorferi* sensu lato in Italy
By: Sophie Melis. University of Pavia, Italy *PhD Student*
- **P28:** Molecular investigation of hemoparasites of the order Piroplasmida in capybaras from endemic and non-endemic areas for Brazilian Spotted Fever in Brazil
By: Thiago Dutra Dias. Universidade Federal Rural do Rio de Janeiro, Brazil *PhD Student*
- **P29:** Vector abundance and associated abiotic factors that influence the distribution of ticks in six provinces of South Africa
By: Tsireledzo Makwarela Goodwill. University of South Africa *PhD Student*
- **P30:** New patterns in seasonal activity of two most common ticks in Slovakia *Ixodes ricinus* and *Dermacentor reticulatus* and its infection with tick-borne agents
By: Veronika Rusňáková Taragelová. Institute of Zoology Slovak Academy of Sciences, Slovakia
- **P31:** The role of birds in the dispersal of ticks and other arthropod vectors
By: Keve Gergő. University of Veterinary Medicine Budapest, Hungary
- **P32:** Citizen science monitoring of tick contact areas in Finland – Lessons, results and uses for data
By: Jani Sormunen. University of Turku, Finland
- **P33:** Vector role of Hyalomma ticks: Comparison of prevalence of infection between ticks collected from two CCHFV-endemic countries (Mongolia and Iraq)
By: VWiktoria Romaneká. University of Warsaw, Poland *PhD Student*
- **P34:** First detection of *Ixodiphagus hookeri* (Hymenoptera: Encyrtidae) in *Ixodes ricinus* ticks (Acari: Ixodidae) in Hungary
By: Adrienn Gréta Tóth. Centre for Bioinformatics, University of Veterinary Medicine, Hungary *PhD Student*
- **P35:** Tick-borne pathogens detected in ticks collected from migratory birds in Sardinia, Italy
By: Valentina Chisu. Istituto Zooprofilattico Sperimentale, Italy

SCIENTIFIC PROGRAM

Thursday September 5th / Meliá Internacional Varadero

■ **8:30-9:30:** The Presence of Tick Salivary Proteins in *Babesia duncani*

By: CEO. Lee Fuller-Fuller Laboratories, USA

Plenary lecture

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero



Chairman

PhD. Sara Moutailler - ANSES, France

■ **9:30-10:00:** High-throughput nanotechnologies for tick-borne pathogens detection

By: Sara Moutailler. ANSES, France

Key talk

■ **10:00-10:20:** *Midichloria mitochondrii* stimulates the sylvatic cycle of Lyme spirochetes in *Ixodes ricinus* instars

By: Hein Sprong. National Institute for Public Health and the Environment, Netherlands

■ **10:20-10:40:** Tick-borne parasites in a high-throughput analysis era: usefulness of MALDI-TOF and Real-Time Microfluidic PCR in the study of parasite distribution in urban wildlife

By: Veronica Risco Castillo. Veterinary School of Alfort, France

■ **10:40-11:00:** Occurrence and genetic identity of *Babesia* spp. in deer and cats in Poland

By: Anna Bajer. University of Warsaw, Poland

■ **11:00-11:30:** Coffee break

■ **11:30-11:50:** First detection of Crimean–Congo haemorrhagic fever virus in *Hyalomma marginatum* ticks, southern France

By: Célie Bernard. CIRAD UMR ASTRE, France

■ **11:50-12:10:** Detection of *Ixovirus* spp. in ticks collected from mink in Spain.

By: Ana M. Palomar. Hospital Universitario San Pedro-CIBIR (Fundación Rioja Salud), Spain

■ **12:10-12:30:** One health in indigenous territories: dogs as sentinels for tick-borne diseases

By: Liliane Silva Durães. Fuller Laboratories, USA

■ **12:30-12:50:** Molecular detection and characterization of tick-borne Anaplasmatidae agents in vampire bats in the Brazilian Amazon

By: Marcos Rogério André. UNESP, Campus de Jaboticabal, Brazil

SCIENTIFIC PROGRAM

- **12:50-13:10:** Prevalence and predictors of tick-borne pathogens in deer communities
By: Maya Holding. UK Health Security Agency, United Kingdom
- **13:10-13:30:** Molecular detection of *Rickettsia*, *Ehrlichia* and *Anaplasma phagocytophilum* in small mammals and ticks from companion animals in Hualien, Taiwan
By: Kun-Hsien Tsai. National Taiwan University
- **13:30-15:00:** Lunch
- **15:00-15:20:** Molecular survey of vector-borne bacterial agents in lowland tapirs (*Tapirus terrestris*) from Brazil
By: Marcos Rogério André. UNESP, Campus de Jaboticabal, Brazil
- **15:20-15:40:** *Neoehrlichia mikurensis* in ticks parasitizing cave-dwelling bats
By: Snežana Tomanović. University of Belgrade, Serbia
- **15:40-16:00:** A novel modeling tool for Rocky Mountain spotted fever intervention
By: Janet Foley. Univ of California, USA
- **16:00-16:15:** Tick-borne pathogens in Southern Norway: Seasonal variation of TBEV, *Borrelia burgdorferi* and *Neoehrlichia mikurensis*
By: Andrea Paola Cotes Perdomo. University of South-Eastern Norway *PhD Student*
- **16:15-16:30:** Diversity and molecular characterization of hemoplasmas infecting bats from the amazon rainforest regions of acre, Brazil
By: Isaac Leandro Lira Pinto. Federal Rural University of Rio de Janeiro, Brazil *PhD Student*
- **16:30-16:45:** Spanish Collaborative Project on Tick and Tick-Borne Pathogens Distribution (GARES)
By: A. Sonia Olmeda. Universidad Complutense de Madrid
- **16:45-17:00:** Elimination of *Babesia ovis* from experimentally infected sheep: Significance of these animals for *Rhipicephalus bursa* larvae in a one-year season
By: Sezayi Ozubek. Firat University, Turkey *PhD Student*

SCIENTIFIC PROGRAM

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero



Chairman

Prof. Petr Kopacek Czech Academy of Sciences, České Budějovice, Czechia

- **9:30-10:00:** Proteases associated with the apical complex of *Babesia*
By: Daniel Sojka. Biology Centre CAS, Czech Republic
- **10:00-10:30:** Advancing visualization and monitoring of tick-borne encephalitis virus infection using a novel reporter system
By: Daniel Ruzek. Masaryk University, Czech Republic
- **10:30-11:00:** Exploring early interactions of lyme disease spirochetes with cells in skin: Insights from volume electron microscopy
By: Marie Vancová. Biology Centre CAS, Czech Republic
- **11:00-11:30:** Coffee break
- **11:30-12:00:** Interactome and forces guiding barrier transmigration of lyme disease *Borrelia*
By: Martin Strnad. University of South Bohemia in České Budějovice, Czech Republic
- **12:00-12:15:** Vector capacity of *Rhipicephalus bursa* for the transmission of *Babesia aktasi* n. sp. infecting for goats
By: Mehmet Can Uluçeşme. Firat University, Turkey PhD Student
- **12:15-12:35:** Co-infection dynamics of *Borrelia afzelii* and TBEV in C3H mice
By: Stefania Porcelli. UMR BIPAR- Ecole Vétérinaire D'Alfort (EnVa), France
- **12:35-12:55:** Impairment of the host immune system impacts transmission and abundance of *Borrelia burgdorferi* in immature *Ixodes scapularis* ticks PhD Student
By: Cody Koloski. University of Saskatchewan, Canada PhD Student
- **12:55-13:15:** Potential leucine aminopeptidase inhibitors of *Haemaphysalis longicornis* (HILAP)
By: Susana Alberti Ramos. Centre for Proteins Studies, Havana University, Cuba PhD Student
- **13:15-13:30:** Evaluating the Pathogenicity of *Babesia aktasi* n. sp. through experimental Infection in Saanen Goats and Sheep
By: Mehmet Can Uluçeşme. Firat University, Turkey PhD Student
- **13:30-15:00:** Lunch



CUBA - 2024

11TH TTP
CONFERENCE

Thursday September 5th

Pathogenesis of
ticks and tick-borne
pathogens

SCIENTIFIC PROGRAM

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

Posters (17:00-18:00)

Posters of all symposia will be presented on Monday September 2nd and Tuesday September 3rd in the room Centro of the Meliá Cohiba hotel

- **P36:** *Babesia bigemina* enolase: a plasminogen-binding protein that enhances plasminogen activation and induces neutralizing antibodies to the infection of in vitro erythrocyte culture
By: Ana Laura Luna Rodríguez. Universidad Autónoma de la Ciudad de México **PhD Student**
- **P37:** Norwegian distributional changes of tick-borne encephalitis virus in a nutshell
By: Ashild Kristine Andreassen. Norwegian Institute of Public Health, Norway
- **P38:** Evaluation of tick-borne parasites in blood smears of wildlife collected during an anthrax outbreak in the Kruger National Park, South Africa
By: Ayesha Hassim. University of Pretoria, Department of Veterinary Tropical Diseases, South Africa
- **P39:** Assessment of tick diversity and potential pathogen transmission in two ecological niches: Implications for zoonotic disease surveillance
By: Elinam Adzo Agbobli. Noguchi Memorial Institute for Medical Research, Ghana
- **P40:** Borreliosis on the Crimean Peninsula
By: Elizaveta Ageeva. Crimean Federal University named after V.I. Vernadsky, Russia
- **P41:** Tick-borne pathogens detected in ticks collected from migratory birds in Sardinia, Italy
By: Gaia Muroli. Istituto Zooprofilattico Sperimentale della Sardegna, Italy
- **P42:** TBEV interaction with human microglia
By: Martin Palus. Biology Centre CAS, Czech Republic
- **P43:** A shared pathogen: *Babesia rossi* in domestic dogs, black-backed jackals (*Canis mesomelas*) and African wilddogs (*Lycaon pictus*) in South Africa
By: Paul Tshepo Matjila. University of Pretoria, South Africa
- **P44:** Unveiling pathogen interactions in Hyalomma Ticks: Insights from central Algerian Steppe Regions
By: Salma Kaoutar Abdelali. University of ferhat abbas – setif – Algeria
- **P45:** Comparative analysis of bovine blood microbiome in two provinces of South Africa using 16S rRNA PacBio approach
By: Zamantungwa Mnisi. University of Limpopo, South Africa

SCIENTIFIC PROGRAM

Symposium Diagnosis and strategies for control of ticks and tick-borne pathogens including immunity and vaccines Room Guamá, Meliá Internacional Varadero



Chairman

PhD. Alina Rodriguez Mallon, Center for Genetic Engineering and Biotechnology, Cuba

- **9:30-10:00:** Acaricide resistance: From genomics to a field-based genotyping assay
By: Michel Labuschagne. Director of Research Innovation, Clinglobal
- **10:00-10:15:** Identification of tick antigens after vaccination with extracellular vesicles in white-tailed deer
By: Adela Oliva. University of Wisconsin, USA
- **10:15-10:30:** In silico identification and chemical remodelling of tick protein epitopes for vaccine antigen development
By: Ingrid Dijkgraaf. Maastricht University, Netherlands
- **10:30-10:45:** The Translationally Controlled Tumor Protein (TCTP) of *Babesia bovis* induces neutralizing antibodies and participates in the establishment of an acute infection
By: Chyntia Quetzalli Pérez Almeida. Universidad Nacional Autónoma de México ^{PhD Student}
- **10:45-11:00:** Next generation tick & tick-borne disease control strategies in Uganda: Preparing for the post-acaricide era!
By: Joseph Byaruhanga. Research Center for Tropical Diseases and Vector Control (RTC), Makerere University, Uganda
- **11:00-11:30:** Coffee break
- **11:30-11:45:** The Tick Cell Biobank – fifteen years of this unique research resource
By: Catherine Hartley. University of Liverpool, United Kingdom
- **11:45-12:00:** Unlocking Bacteriophages: Pioneering Diagnostics and Marker Discovery for early diagnosis of Lyme and tick-borne diseases
By: Jinyu Shan. University of Leicester, United Kingdom
- **12:00-12:15:** Acaricide resistance: The performance of acaricides active ingredients and acaricide formulations against tick treatments in bioassay tests
By: Faith Mutavi. Wageningen University and Research ^{PhD Student}
- **12:15-12:30:** Changes in the serum proteome profile of patients with neuroborreliosis, foresters, and patients treated according to ILADS method
By: Anna Moniuszko. Malinowska -Medcial University in Białystok, Poland

SCIENTIFIC PROGRAM

- **12:30-12:45:** Exploration of emerging tick-borne pathogens and neglected tick-borne diseases in Serbia: Balkan model of One Health approach
By: Pavle Banović. Pasteur Institute Novi Sad, Serbia and Montenegro
- **12:45-13:00:** Association of the bovine leukocyte antigen major histocompatibility complex exon II DRB3*020:02:01 to host resistance to *Theileria orientalis* infection in crossbred Kedah-Kelantan cattle
By: Onyinyechukwu Ada Agina. University of Nigeria
- **13:00-13:15:** Effects of Ash on *Ixodes pacificus* and *Dermacentor* Spp. Survival, Behavior, and Host Interaction
By: Francesca Rubino. University of California, USA
- **13:15-13:30:** Screening for immune biomarkers associated with infection or protection against *Ehrlichia ruminantium* by RNA-sequencing analysis
By: Tshifhiwa Nefefe. Agricultural Research Council – Onderstepoort Veterinary Research, South Africa
- **13:30-15:00:** Lunch

Posters (17:00-18:00)

Posters of all symposia will be presented on Monday September 2nd and Tuesday September 3rd in the room Centro of the Meliá Cohiba hotel

- **P46:** Identification of peptides containing B-cell epitopes of the VDAC and RI-86 proteins of *Rhipicephalus linnaei* and assessment of their immunogenicity
By: Aldo Josué Pavón Rocha. Universidad Autónoma de Querétaro, Mexico *PhD Student*
- **P47:** Identification of biomarkers after *Ixodes ricinus* tick bite exposure as a diagnostic and surveillance tool
By: Alexis Dziedzic. Institute Pasteur, France
- **P48:** In silico methodologies combined in vivo assessment of immunogenicity to uncover *Rhipicephalus bursa* – *Babesia ovis* interactions
By: Ana Gonçalves Domingos. IHMT – Universidade NOVA de Lisboa, Portugal
- **P49:** Production of monoclonal antibodies against *Babesia ovis* relevant proteins
By: Ana Gonçalves Domingos. IHMT – Universidade NOVA de Lisboa, Portugal
- **P50:** Dissecting Hazara virus-tick dynamics: the role of viral-derived DNA forms in *Hyalomma marginatum*
By: Ana Isabel Amaro Gonçalves Domingos. IHMT – Universidade NOVA de Lisboa, Portugal
- **P51:** Vaccinomics-based selection and validation of protective salivary antigens from *Ornithodoros moubata*
By: Ana Oleaga Pérez. Instituto de Recursos Naturales y Agrobiología de Salamanca (CSIC), Spain

SCIENTIFIC PROGRAM

- **P52:** Microfluidic PCR and network analysis reveals complex tick-borne pathogen interactions in the tropics
By: Belkis Corona González. CENSA, Cuba
- **P53:** Mediterranean spotted fever on the Crimean Peninsula
By: Elizaveta Ageeva. Crimean Federal University named after V.I. Vernadsky, Russia
- **P54:** PCR targeting the large subunit of phage terminase – a suitable tool for *Borrelia* diagnostic?
By: Gabriele Margos. Bavarian Health and Food Safety Authority, Germany
- **P55:** Real Time PCR for Detection of *Rickettsia* spp. in *Rhipicephalus sanguineus* collected from areas of extreme poverty in Peru
By: Giovanna Mendoza Mujica. Instituto Nacional de Salud Lima Perú
- **P56:** Seroprevalence of tick-borne encephalitis in small ruminants and dogs in the Czech Republic and experimental veterinary vaccine development
By: Jiri Salat. Veterinary Research Institute, Czech Republic
- **P57:** High seroprevalence of *Bartonella henselae* in healthy blood donors in the Czech Republic
By: Katerina Kybicova. National Institute of Public Health, Czech Republic
- **P58:** Genome-wide analysis of *Theileria parva* proteases for identification of potential drug targets
By: Kgomotso Sibeko Matjila. University of Pretoria, South Africa
- **P59:** Molecular detection and epidemiological analysis of *Anaplasma marginale* in *Rhipicephalus microplus*, *Stomoxys calcitrans* and *Haematobia irritans*
By: Laís Feliciano de Souza. Federak Rural University of Rio de Janeiro, Brazil
- **P60:** Conventional multiplex PCR for detection of tick-borne pathogens: proof of concept and utility in biological sample
By: Laurine Levillayer. Institut Pasteur Paris, France
- **P61:** Generation of a recombinant single variable domain (VHH) antibody from the Lama glama model against a *Babesia bovis* antigen.
By: Mayra Mirelle Becerra Reyes. Universidad Autónoma de Querétaro, Mexico PhD Student
- **P62:** Evaluation of integrated One Health economic policies to control Crimean Congo Hemorrhagic Fever
By: Muhammad Asaduzzaman. University of Oslo, Norway
- **P63:** IRIXIN – a novel contact phase coagulation inhibitor from the tick *Ixodes ricinus*
By: Radek Sima. Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Czech Republic PhD Student
- **P64:** Integrated Approach for Assessing Tick-Borne Pathogens in South African Horses: Quantitative Detection of *Theileria haneyi* and Molecular Genotyping of *Babesia caballi*
By: Raksha Vasantrai Bhoora. University of Pretoria, South Africa



CUBA - 2024

11TH TTP
CONFERENCE

Thursday September 5th

Diagnosis and strategies for control of ticks and tick-borne pathogens including immunity and vaccines

SCIENTIFIC PROGRAM

- **P65:** Development of cocktail vaccines against *Ornithodoros argasid* ticks
By: Ricardo Pérez-Sánchez. (CSIC)- (IRNASA), Spain
- **P66:** *Ehrlichia* Species in Dromedaries and Ruminants from Somalia: With the First Report of *Ehrlichia minasensis* in Dromedaries, Sheep, and Goats Globally
By: Rosangela Zacarias Machado. FCAV/UNESP, Brazil
- **P67:** In vitro evaluation of OleoVET and formulations action on reproductive indicators of teleogins (*Rhipicephalus microplus*)
By: Roxana Gómez Zaldivar. CNIC, Cuba *PhD Student*
- **P68:** Establishment of a fluorescence-based method for anti-*Babesia ovis* drug screening
By: Sandra Isabel Da Conceição Antunes. IHMT – Universidade NOVA de Lisboa, Portugal
- **P69:** Diagnostic difficulties in a clinical case of tuberculous meningo-encephalitis complicated by superinfection with West Nile virus
By: Costache Delia. Faculty of Medicine, Transylvania University Brasov, Romania
- **P70:** Loop-mediated isothermal amplification (LAMP) assay for rapid visual detection of *Anaplasma marginale* infection in bovines CIGB, Cuba
By: Lianet Abuin Denis. CIGB, Cuba *PhD Student*
- **P71:** Obtention of recombinant msp5 antigen to develop an ELISA for *Anaplasma* sp. diagnostic
By: Marisdania Joglar Piñeiro. CIGB, Cuba
- **P72:** Amplification of microsatellites of *Rhipicephalus sanguineus* ticks using specific primers from *Rhipicephalus microplus* ticks.
By: Anailí Ávila Espronceda. CIGB, Cuba *PhD Student*
- **P73:** A novel method for determining the efficacy of acaricides under field trial conditions
By: Joseph Byaruhanga. Research Center for Tropical Diseases and Vector Control (RTC), Makerere University. Uganda
- **P74:** Enhancing East Coast Fever Vaccination: The Potential of TLR Agonists as Adjuvants
By: Harriet Matildah Oboge. University of Nairobi, Kenya

SCIENTIFIC PROGRAM

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero



Chairman

PhD. Ala Tabor, The University of Queensland, Australia

- **15:00-15:25:** Evaluation of the Effectiveness of the GAVAC® Vaccine and Rational Use of Acaricides as an Alternative in an Integrated Tick Control Program in Ecuador
By: Richard Rodríguez. Universidad Central del Ecuador
- **15:25-15:50:** Current status of vaccination against the cattle tick *Rhipicephalus microplus* in Mexico
By: Consuelo Almazán. Autonomous University of Queretaro, Mexico
- **15:50-16:15:** Immunization of cattle with *Rhipicephalus microplus* voraxin peptides, which contain predicted B-cell epitopes, decreases tick fitness
By: Juan Mosqueda. Autonomous University of Queretaro, Mexico
- **16:15-16:40:** Anti-tick vaccines and commercial challenges
By: Alicja (Ala) Elzbieta Tabor. The University of Queensland, Australia
- **16:40-17:00:** Change of mentality when it comes to anti-tick vaccine commercialization
By: Alina Rodríguez Mallon. CIGB, Cuba

Posters (17:00-18:00)

Posters of all symposia will be presented on Monday September 2nd and Tuesday September 3rd in the room Centro of the Meliá Cohiba hotel

- **P75:** Optimizing tick vaccines with multi-antigenic formulations
By: Beatriz Rossetti Ferreira. Universidade de São Paulo
- **P76:** Implementation of orthogonal methods to facilitate conjugation site assignment in conjugate vaccines against tick infestation
By: Pablo E. Ramos-Bermúdez. CIGB, Cuba ^{PhD Student}
- **P77:** Characterization by LC-MS/MS of tick vaccine conjugates based on p0 peptide.
By: Luis J. González. CIGB, Cuba
- **P78:** BMPS as a promising cleavable crosslinker for the development and characterization of vaccine conjugates against ticks ^{PhD Student}
By: Satomy Pousa. CIGB, Cuba
- **P79:** Efficacy of anti-tick vaccine based on the P0 peptide against *Ixodes ricinus* and *Dermacentor nitens* ticks
By: Frank Luis Ledesma Bravo. CIGB, Cuba ^{PhD Student}

SCIENTIFIC PROGRAM

- **P80:** Demonstrating consistency in quality control of the Gavac® vaccine, a success story
By: Alain Moreira Rubio. CIGB Camagüey, Cuba
- **P81:** Technological update of fermenters and increase in scale in the production of Gavac® Biomass
By: Helder Álvarez Martínez. CIGB Camagüey, Cuba *PhD Student*
- **P82:** Proposal for scaling up the chemical conjugation process of the P0 peptide to the Bm86 protein for the production of a broad-spectrum tick vaccine
By: Ernesto Álvarez Zaldivar. CIGB Camagüey, Cuba
- **P83:** Obtaining, development and sanitary registration of the veterinary diagnostic product HeberFast Line® GAVAC for use in field conditions
By: Dayamí Dorta. CIGB of Sancti Spiritus, Cuba
- **P84:** Results of vaccination with Gavac within an Integrated Tick Control Program in Cuba
By: Mara Laura Hernández García. CIGB, Cuba *PhD Student*
- **P85:** Enhancing data management efficiency in the integrated vaccination program with GAVAC: A proposal for a multiplatform application
By: Julio E. Duque Vizcaíno. CIGB, Cuba *PhD Student*

Friday September 6th / Meliá Internacional Varadero

- **8:30-9:30:** Ticked off: tackling the threat of *Rhipicephalus microplus* in cattle
By: PhD. Maxime Madder- Director of Parasitology and Vector Borne Diseases. Clinglobal

Plenary lecture

Symposium Taxonomy and evolution of ticks and tick – borne pathogens Room Internacional I, Meliá Internacional Varadero



Chairman

PhD. Filipe Dantas Torres, Aggeu Magalhães Institute, Brazil

- **9:30-10:00:** The *Rhipicephalus sanguineus* group taxonomy: unclosed gaps
By: Filipe Dantas Torres. Aggeu Magalhães Institute, Brazil
- **10:00-10:20:** Perspectives on Argasid Evolution
By: Ben J Mans. Agricultural Research Council-Onderstepoort Veterinary Research, South Africa
- **10:20-10:40:** Nuttalliellidae in Burmese amber: Implications for tick evolution
By: Chitimia-Dobler Lidia. Fraunhofer Institute of Immunology, Infection and Pandemic Research, Germany
- **10:40-11:00:** Discovery of a novel Mediterranean *Haemaphysalis (Ornithophysalis) doenitzi* group tick species infesting *Falco eleonora* on Antikythira Island, Greece
By: Anastasios Saratsis. Veterinary Research Institute/Hellenic Agricultural Organisation DIMITRA, Greece

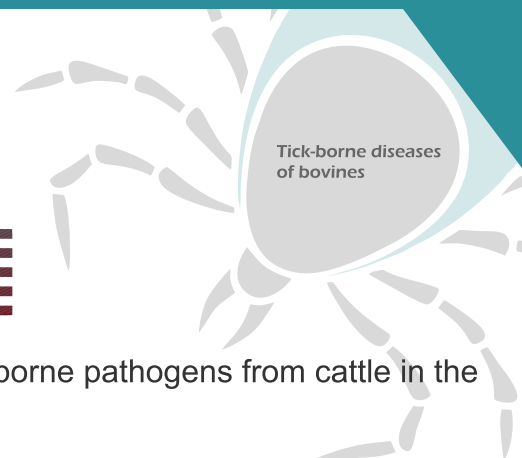
SCIENTIFIC PROGRAM

- **11:00-11:30:** Coffee break
- **11:30-11:50:** Fragments of 12s and 16s mitochondrial genes are useless when compared to fragments of cox 1 in tick taxonomy and diagnosis
By: Stephen C. Barker (Recorded). University of Queensland, Australia
- **11:50-12:10:** *Rhipicephalus sanguineus* s.l transcriptome analysis along with instar from four natural regions of Colombia
By: Gabriel Andres Tafur Gómez. Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA, Mosquera 250047, Colombia
- **12:10-12:30:** A MALDI-TOF MS approach to differentiate *Amblyomma maculatum* ticks of public health importance in the United States
By: Maria Fernanda Bandeira de Melo Galletti. Centers for Disease Control and Prevention, USA
- **12:30-12:50:** Integrated approaches for surveillance of ticks and tick-borne diseases (Arachnida: Ixodidae) of livestock importance
By: Luis Miguel Hernandez Triana. Animal and Plant Health Agency, United Kingdom
- **12:50-13:10:** A core genome MLST scheme provides higher resolution insights into the *Borrelia burgdorferi* sensu lato species complex
By: Sabrina Hepner (Recorded). Bavarian Health and Food Safety Authority, Germany
- **13:10-13:30:** *Borrelia tillae* – revival of a *Borrelia* species from South Africa
By: Gabriele Margos (Recorded). Bavarian Health and Food Safety Authority, Germany
- **13:30-15:00:** Lunch

Posters (17:00-18:00)

Posters of all symposia will be presented on Monday September 2nd and Tuesday September 3rd in the room Centro of the Meliá Cohiba hotel

- **P86:** Description of ticks (Acari: Ixodidae) from Dominican amber below consideration of the recent genus *Amblyomma*
By: Chitimia Dobler Lidia. Fraunhofer Institute of Immunology, Infection and Pandemic Research, Germany
- **P87:** Combining morphological and molecular approaches to improve the systematic of the genus *Hyalomma* Koch, 1844
By: A. Sonia Olmeda. Universidad Complutense de Madrid, Spain
- **P88:** Molecular characterization of *Ehrlichia canis* TRP36 in thrombocytopenic dogs from Rio de Janeiro, Brazil
By: Bruna de Azevedo Baêta. Universidade Federal Rural do Rio de Janeiro, Brazil
- **P89:** The European badger (*Meles meles*) as a host for ticks and tick-borne pathogens in peri-urban environments
By: Sándor Szekeres. University of Veterinary Medicine, Budapest, Hungary



SCIENTIFIC PROGRAM

- **P90:** Phenotypic and genotypic characterization of ticks and tick-borne pathogens from cattle in the villages
By: Katileho Sechaba Monakale. University of South Africa *PhD Student*
- **P91:** Description of the larva of *Dermacentor latus* Cooley, 1937 (Ixodida: Ixodidae) from two localities in Costa Rica
By: Luis Enrique Chaves-González. Universidad de Costa Rica (UCR) *PhD Student*
- **P92:** A systematic review of ticks and tick-borne pathogens of cattle reared by smallholder farmers in South Africa
By: Petunia Malatji. University of South Africa
- **P93:** Unveiling the genome of *Babesia ovis* Israeli strain: empowering *Babesia* research
By: Sandra Isabel Da Conceição Antunes. IHMT, Portugal
- **P94:** Non-destructive extraction of DNA: a method to keep the shape !
By: Lorang C. ANSES, France *PhD Student*
- **P95:** Assessing risk factors and tick-borne pathogens in grazing cattle of northeastern Colombia
By: Gabriel Andres Tafur Gómez. Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA, Mosquera 250047, Colombia

Symposium Tick-borne diseases of bovines Room Internacional II, Meliá Internacional Varadero



Chairmen

PhD. Consuelo Almazán & Prof. Juan Mosqueda, Autonomous University of Queretaro, Mexico

- **9:30-10:00:** Tick borne pathogens in ruminants horses and ticks in Croatia
By: Relja Beck. Croatian Veterinary Institute
- **10:00-10:30:** The roles of vitellogenins and their related molecules on *Babesia* transmission in *Haemaphysalis longicornis* ticks
By: Rika Umemiya Shirafuji. Obihiro University of Agriculture and Veterinary Medicine, Japan
- **10:30-11:00:** Abundance and distribution of *Rhipicephalus microplus* complex infesting *Bos indicus* animals from 10 cattle farms in Cambodia
By: Sony Yean. Institut Pasteur du Cambodge, Cambodia *PhD Student*
- **11:00-11:30:** Coffee break

SCIENTIFIC PROGRAM

Posters (17:00-18:00)

Posters of all symposia will be presented on Monday September 2nd and Tuesday September 3rd in the room Centro of the Meliá Cohiba hotel

- **P96:** Coinfection of *Anaplasma bovis*, *Anaplasma* spp. and *Theileria orientalis* complex in European bison (*Bison bonasus*)
By: Ana M. Palomar. Hospital Universitario San Pedro-CIBIR (Fundación Rioja Salud), Spain
- **P97:** Molecular detection and epidemiological analysis of *Anaplasma marginale* in *Rhipicephalus microplus*, *Stomoxys calcitrans* and *Haematobia irritans*
By: Laís Feliciano de Souza. Universidade Federal Rural do Rio de Janeiro, Brazil ^{PhD Student}
- **P98:** Insights and methodological approaches for exploring hemoglobin metabolism in *Babesia* parasites
By: Viktoriya Levytska. Institute of Parasitology, Biology Centre CAS, Czech Republic

Symposium Tick microbiome and genomics Room Internacional I, Meliá Internacional Varadero



Chairman

PhD. Ana Gonçalves Domingos, IHMT, Universidade NOVA de Lisboa, Portugal

- **11:30-11:50:** Internal organ metagenomics of adult semi engorged *Rhipicephalus australis* female ticks
By: Alicja (Ala) Elzbieta Tabor. The University of Queensland, Australia
- **11:50-12:10:** RNA Virome of *Ixodes ricinus* in Slovenia
By: Natasa Knap. University of Ljubljana, Slovenia
- **12:10-12:30:** A multiplex, amplicon based nanopore sequencing assay for characterisation of UK tick species, their microbiomes and bloodmeal origin
By: Mia White. UKHSA, UK
- **12:30-12:50:** Metagenomic analysis of *Rickettsia* plasmid sequences obtained directly from ticks
By: Gregory A. Dasch. CDC, USA
- **12:50-13:10:** Bacterial microbiota of ticks infesting humans in the Yozgat province of Türkiye
By: Kosta Mumcuoglu. The Hebrew University of Jerusalem, Israel
- **13:10-13:30:** Microbiome of the bush tick (*Haemaphysalis longicornis*): the current state of play revisited
By: Abdul Ghafar. University of Melbourne, Australia
- **13:30-15:00:** Lunch

SCIENTIFIC PROGRAM

Posters (17:00-18:00)

Posters of all symposia will be presented on Monday September 2nd and Tuesday September 3rd in the room Cetro of the Meliá Cohiba hotel

- **P99:** Investigating the role of miRNAs in the salivary glands of the soft ticks *Ornithodoros moubata* and *Ornithodoros erraticus*
By: Ana Laura Cano Argüelles. Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA), Spain **PhD Student**
- **P100:** Detection of multiple novel viruses in hard and soft ticks in Mexico
By: Javier Alfonso Garza Hernández. Universidad Autónoma de Ciudad Juárez, Mexico
- **P101:** Tick genomics – Progress towards tick genome assembly and annotation best practices
By: Katie Dillon. University of Georgia, USA **PhD Student**
- **P102:** Karoo Paralysis tick (*Ixodes rubicundus*) salivary gland transcriptomes – in search of a toxin
By: Ronel Pienaar. ARC-Ondertsepoort Veterinary Research, South Africa
- **P103:** Bacteriome Diversity in *Babesia ovis*-Infected *Rhipicephalus bursa* salivary glands and midguts
By: Sandra Isabel Da Conceição Antunes. IHMT, Portugal
- **P104:** Understanding the vertical and horizontal transmission potential of *Spiroplasma* in ticks
By: Shohei Ogata. Hokkaido University, Japan



CUBA - 2024

11TH TTP
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PLENARY LECTURES

Sunday September 1st / Meliá Cohiba



Schedule: 17:30-18:30

Title: The role of agricultural biotechnology in the One Health Commitment

By: PhD. Mario Pablo Estrada Garcia – CIGB, Cuba – Director of Agricultural Researches

The agricultural biotechnology gives to the food producers tools to produce more cheap, friendly environment, healthy, managerial and profitable. Genetic improvement of plants and animals, the creation of platforms to combat the emergent diseases, the improvement of nutritional and quality of food, the use of new technology among other important issues are tools for the “management of knowledge” in function of food production. The facts of world increase population, urban migration and the climate change, are three mandatory variables to be in consideration for any strategic plan for the future in the food production. The transition time between the experiment in tubes in our laboratories to the final products to do an impact in the society, must be reduced, with the commitment to work in the next years for One Health as an approach that recognizes that the health of people is closely connected to the health of animals and our shared environment. The people of science have a huge challenge using all the data available, the new discoveries in the science of life, to fully fill the food demand and nutritional quality, for the next generations. In this presentation we are sharing examples from the molecular biology to a final product with their impact in the life of the people.



CUBA - 2024

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PLENARY LECTURES

Sunday September 1st / Meliá Cohiba



Schedule: 18:30-19:30

Title: Dynamics of tick feeding and why it matters

By: Prof. Pat Nuttall-Department of Biology, University of Oxford, UK

Imagine feeding on an elephant. From sensing the elephant's presence, climbing onboard, and searching for a good site to dig in, it takes an additional 10 days or more for an adult female elephant tick, *Amblyomma tholloni*, to fully engorge and then drop off into the vegetation to lay eggs. What happens during these 10 days of blood-feeding? We know little of the habits of the elephant tick but studies on other ixodid species are providing insights. Basically, tick feeding is extremely complicated – a neural network of interactions between the tick, its host, and their respective microbiomes (including tick-borne pathogens). Different nodes of this network are active at different times during feeding. Unravelling the hidden layers of these interactions to understand what is happening during tick blood-feeding will most likely require the help of artificial intelligence. Nevertheless, it is possible to examine the significance of 'time' by breaking down the feeding period into sequential phases: (i) initiation, (ii) attachment, (iii) slow feeding, (iv) mating, (v) rapid feeding, and finally, (v) detachment. What happens at each phase – to the tick, the host, and any tick-borne pathogens caught up in the mix? This conceptualization of feeding dynamics helps explain why it is important to consider the time dimension when studying tick feeding: to understand the conditions when tick-borne pathogens are transmitted (from tick to host and vice versa), identify vulnerabilities when interventions may be most effective, and optimise strategies for controlling ticks and tick-borne infections. One day, we might even help elephants suffering irritating tick infestations; in the meantime, they must scratch their itch.



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PLENARY LECTURES

Monday September 2nd / Meliá Cohiba



Schedule: 8:30-9:30

Title: Kill the Tick' – an endless gameplay one never gets tired of

By: Prof. Petr Kopáček- Czech Academy of Sciences, České Budějovice, Czechia

Ticks are disgusting creatures and dangerous vectors of pathogens that cause serious diseases in humans and animals. And yet these parasites are highly appealing and interesting when viewed open under a microscope or dissected in a test tube. In Czechia where I live, ticks, more precisely *Ixodes ricinus* (the vector of Lyme disease and tick-borne encephalitis), are fortunately the only remaining important ectoparasites. All the more reason for them to attract the attention of research scientists and laypeople alike. These circumstances brought me to tick research about a quarter of a century ago. The first question I asked myself, coming from the field of invertebrate immunity, was: "How can tick-borne pathogens evade the innate immune response of ticks?" Gradually, we found out that unlike insects or crustaceans, ticks do not have a prophenoloxidase-activating cascade that leads to melanization, but have a highly conserved ancient complement system. Another interesting question that arose some time later was: "How can ticks ingest the extreme amount of host blood, digest it and process it into a huge clutch in a very short time?" Processing the blood meal led to another question: "What do ticks do with the excessive amount of pro-oxidative heme and iron from the host's hemoglobin?" The surprising scarcity and lack of a core microbiome in the midgut of *I. ricinus* raised the question of the molecules involved in tick midgut immunity. In today's post-genomic era, we are inundated with a wealth of genomic, transcriptomic and proteomic datasets, and it is quite a challenge to extract relevant and valid information from them. Our task is to further decipher the function of selected molecules or entire signaling pathways using the tools of functional genomics such as RNA interference or experimental membrane feeding. In the future, we will focus on another fascinating feature of tick physiology – the ability of ticks to survive long-term starvation between feeding episodes on their hosts. This topic is closely related to the use of lipid stores, nutrient sensing and signaling. A better understanding of the molecular physiology of ticks and the mechanisms that facilitate the transmission of tick-borne pathogens will hopefully help us to reach higher levels in the 'Kill the Tick' game.



CUBA - 2024

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PLENARY LECTURES

Tuesday September 3th / Meliá Cohiba



Schedule: 8:30-9:30

Title: What does it cost to be a vector: Effects of tick-borne pathogens on the survival and behavior of ticks

By: Dr. Michael Levin, CDC, Atlanta, USA (Retired)

Symbiosis is defined as any form of a close and long-term biological interaction between members of different species, termed symbionts, be it mutualistic, commensalistic, or parasitic. The relationship between ticks and tick-borne pathogens is an example of symbiosis where the latter species critically depend on the former for their propagation and their very existence. In this presentation, I will assess costs and benefits incurred by ticks carrying three important rickettsial tick-borne pathogens to identify the specific types of symbiotic relationships existing between those pathogens and their respective tick-vectors. To this effect, I will compare life cycle parameters between uninfected ticks and ticks infected with agents of Israeli tick typhus – *Rickettsia conorii*, Rocky Mountain spotted fever – *Rickettsia rickettsii*, and human granulocytic anaplasmosis – *Anaplasma phagocytophilum*. Although no obvious deleterious effects were observed among infected ticks, several of the analyzed parameters decreased in infected ticks indicating antagonistic relationships between the studied tick-borne pathogens and their natural vector. Specifically, infection with *R. rickettsii israelensis* resulted in decreased both nymphal and adult molting success of *Rhipicephalus sanguineus* s.s. ticks as well as the feeding success at the nymphal stage. Infection with *R. rickettsii* prolonged feeding periods of *Dermacentor variabilis* ticks in all life stages. *R. rickettsii*-infected nymphal and adult ticks experienced a slight decrease in feeding success compared with ticks from an uninfected colony, but neither nymphal nor adult molting success was affected. Infected females reached smaller engorgement weights, were less efficient in conversion of bloodmeal into eggs, and produced smaller egg clutches with a lower proportion of eggs hatching. However, the longevity was not decreased due to *R. rickettsii* infection in life any stage. On the other hand, infection with *A. phagocytophilum* decreased the nymphal molting success of *Ixodes scapularis*. It also reduced the longevity of infected *I. scapularis* nymphs. At the same time, individual ticks infected with *A. phagocytophilum* appeared “hungrier” than the uninfected ones as they moved faster and more directly toward a potential host. Also, both the prevalence of *A. phagocytophilum* infection among infected tick cohorts and the average quantity of pathogen in starving ticks decreased over time. Overall, symbiotic relationships existing between the three studied tickborne pathogens and their respective vectors can be classified as parasitic.



CUBA - 2024

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PLENARY LECTURES

Thursday September 5th / Meliá Internacional Varadero



Schedule: 8:30-9:30

Title: A New Look at Rickettsia ELISA assays

By: CEO. Lee Fuller-Fuller Laboratories, USA

We consider Rickettsia ELISA assays first as reference laboratory automatable assays, then also as a methodology to be formatted as rapid tests. Both Spotted Fever (SFG) and Typhus Group (TG) Rickettsia species are amenable to both IgG and IgM testing without the trouble of interpreting IFA slides microscopically. The group-specific lipopolysaccharide (LPS) antigens are excellent substrates for IgG testing of both SFG and TG species due to the early rise in titer during acute phase rickettsiosis. Also, the barrel peptide of rOmpB can be used successfully to determine species-specific results for IgG when relevant. Yet the IgM assays remain the keystone for acute phase diagnosis. Here the rOmpB-associated β -peptide is that specific antigen that binds only immune IgM. It does not bind IgG or non-immune IgM, making it perfect for both ELISA and rapid test formats. Some new properties of this β -peptide are discussed.



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PLENARY LECTURES

Friday September 6th / Meliá Internacional Varadero



Schedule: 8:30-9:30

Title: Ticked off: tackling the threat of *Rhipicephalus microplus* in cattle

By: PhD. Maxime Madder- Director of Parasitology and Vector Borne Diseases. Clinglobal

The presentation will explore the biology, impact, distribution and management of the cattle tick *Rhipicephalus microplus*, a significant ectoparasite affecting livestock worldwide. The session will highlight current distribution and displacement and hybridization with other one-host tick species, control methods, such as chemical acaricides, and their limitations, particularly the rise of tick resistance. Innovative approaches, including integrated pest management strategies and the development of vaccines, will be discussed and the need for standardized models presented. The presentation aims to provide a comprehensive overview of the challenges and advancements in managing *Rhipicephalus microplus* to improve cattle health and agricultural sustainability.



CUBA - 2024

Symposium Tick microbiota

Monday September 2nd, Room Cetro - Meliá Cohiba

9:45-10:30

Effects of *Rickettsia rickettsii* infection on its vector microbiota

Daniel B. Pavanelo^{1#}, Eliane Esteves^{1##}, Solange C. Antão¹, Marcelly B. Nassar¹, Beatriz I. Alonso¹, Sirlei Daffre¹, Marcelo B. Labruna², Ludek Zurek³, Petr Kopáček⁴, Marisa Farber⁵, Andréa C. Fogaça^{1*}

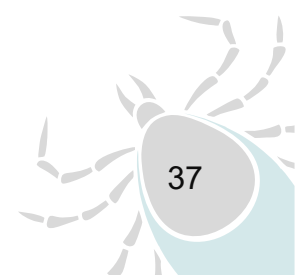
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Present address: #Laboratório Central do Rio Grande do Sul, Centro Estadual de Vigilância em Saúde, Secretaria da Saúde, Rio Grande do Sul, Brazil; ##Department of Microbiology and Immunology, College of Medicine, University of South Alabama, Mobile, AL, United States.

*Prof. Andrea Fogaça (deafog@usp.br)

Amblyomma sculptum and *Amblyomma aureolatum* are vectors of *Rickettsia rickettsii*, the causative agent of Brazilian spotted fever. These two tick species present a conspicuous difference concerning their susceptibility to infection with *R. rickettsii*. *A. aureolatum*, which is highly susceptible to infection, harbors a dense bacterial population in the midgut, mainly composed of bacteria in the *Francisella* genus. Conversely, the midgut of *A. sculptum* is almost sterile, correlating with its low susceptibility to *R. rickettsii*. Similar to *A. sculptum*, a reduced microbiota was also reported to occur in the midgut of other tick species, which harbor a more abundant and stable microbiota in the ovaries. Therefore, we extended the analysis to include additional organs of *A. sculptum* and *A. aureolatum* exposed or not to *R. rickettsii*. A lower number of bacteria was detected in the ovaries and salivary glands of *A. sculptum* in comparison with *A. aureolatum*, as previously observed in the midgut. Although the bacterial load in the midgut of *A. aureolatum* was higher than in *A. sculptum*, this organ harbors fewer bacteria than the salivary glands and ovaries. While higher bacterial loads were detected in the organs of *R. rickettsii*-exposed *A. aureolatum*, non-significant differences were observed in the organs of *A. sculptum* exposed or not to infection. The bacterial community composition was explored using high-throughput sequencing of the 16S rRNA gene V3-V4 hypervariable regions. As previously observed, *Francisella* was the predominant genus in the midgut of nonexposed *A. aureolatum*. In the salivary glands and particularly in the ovaries, the proportion of the genus *Francisella* was even higher than in the midgut. Additional studies are warranted to assess the role played by the components of the microbiota on tick fitness and the acquisition of *R. rickettsii*.

(Supported by FAPESP 2021/03649-4)





CUBA - 2024

Symposium Tick microbiota

Monday September 2nd, Room Cetro - Meliá Cohiba

10:30-11:00

Dynamic nesting of *Anaplasma marginale* in the microbial communities of *Rhipicephalus microplus*

Authors: Elianne Piloto-Sardiñas^{1,2,*}, Lianet Abuin-Denis^{1,3}, Apolline Maitre^{1,4,5}, Angélique Foucault-Simonin¹, Belkis Corona-González², Cristian Díaz-Corona², Lisset Roblejo-Arias², Lourdes Mateos-Hernández¹, Roxana Marrero-Perera², Dasiel Obregon⁶, Karolína Svobodová⁷, Alejandra Wu-Chuang¹, Alejandro Cabezas-Cruz^{1,*}

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The tick microbiome represents a dynamic microecosystem, influenced by complex interactions among pathogenic and non-pathogenic microorganisms alongside external factors. This study delves into the temporal dynamics within the *Rhipicephalus microplus* microbiome, with particular emphasis on its interactions with *Anaplasma marginale*. Employing next-generation sequencing and network analysis, we examine microbiome fluctuations over a span of two years, unveiling profound alterations in diversity, composition, and structure. Among the key findings are the dynamic shifts in microbial diversity, indicating *Anaplasma*'s impact on tick microbiota acquisition and colonization processes. Moreover, our analysis reveals the transition of *Anaplasma* from diverse connections to keystone taxon status, signifying its evolving role in shaping microbiome dynamics. Notably, we identify evidence of "keystone pathogen-induced dysbiosis," suggesting *Anaplasma*'s pivotal role in modulating microbial community assembly. Additionally, through an integrative approach, we demonstrate *Anaplasma*'s significant impact on network stability, highlighting its crucial role in conferring resilience to the microbiome. These insights underscore the critical importance of considering the temporal dimension in tick-microbiome interactions, which is essential for devising targeted strategies to manipulate bacterial communities and mitigate the spread of vector-borne diseases. Our findings present novel avenues for controlling bovine anaplasmosis through microbiome modulation, contributing to the advancement of disease ecology and management strategies in the context of tick-borne illnesses.

KEYWORDS

Rhipicephalus microplus, microbiome dynamics, ticks, nesting, *Anaplasma marginale*, networks



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Oral presentations

Symposium Tick microbiota

Monday September 2nd, Room Centro - Melià Cohiba

11:30-12:00

The potential of anti-microbiota vaccines: a study on the soft tick *Ornithodoros moubata*

Ana Laura Cano-Argüelles¹, Elianne Piloto-Sardiñas^{2,3}, Apolline Maitre^{3,4,5}, Lourdes Mateos-Hernández³, Jennifer Maye⁶, Alejandra Wu-Chuang³, Lianet Abuin-Denis^{3,7}, Dasiel Obregón⁸, Timothy Bamgbose^{9,10}, Ana Oleaga¹, Alejandro Cabezas-Cruz³, Ricardo Pérez-Sánchez¹

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Ornithodoros moubata is the main vector of African swine fever (ASF) and human relapsing fever in South and East Africa. Given the limitations of chemical acaricides, there is an urgent need for innovative approaches to control tick and tick-borne diseases. In this context, anti-microbiota vaccination emerges as a promising strategy to disrupt the physiology of the tick vector and its capacity to transmit pathogens. In this study, we investigated the efficacy of an anti-microbiota vaccine against *O. moubata* in terms of the impact of host antibodies on tick fitness and the midgut microbiome.

We had previously assessed the diversity, taxonomy and co-occurrence networks of the bacterial microbiome of *O. moubata* salivary glands and midgut using 16S rRNA gene sequencing, identifying keystone bacterial taxa in each tissue. Based on these findings, we selected *Pseudomonas*, a keystone taxon, as a target for conducting an anti-microbiota vaccine trial in rabbits. *In silico* analysis revealed that the removal of *Pseudomonas* from microbial co-occurrence networks could lead to changes in the composition and connectivity patterns of tick bacterial communities.

Vaccination with *Pseudomonas* and *Lactobacillus*, a non-keystone taxon used as a control, elicited strong humoral responses in rabbits, with significantly higher levels of IgG antibody in the *Pseudomonas* group. The anti-*Pseudomonas* vaccine led to reductions in female tick survival and fertility, demonstrating a low but significant protective efficacy (7.9%). The microbiome of tick midgut also showed vaccine-induced alterations in taxonomic diversity and microbial community dynamics, emphasizing the importance of selecting the right vaccine target.

Our study offers insights into the intricate dynamics of *O. moubata* microbial networks and underscores the potential utility of targeted anti-microbiota vaccines for tick control.

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CUBA - 2024

Symposium Tick microbiota

Monday September 2nd, Room Centro - Meliá Cohiba

12:00-12:30

Host microbiota-induce antibodies alter tick microbiota and block *Borrelia* colonization in ticks

Lourdes Mateos-Hernandez^{1,†}, Alejandra Wu-Chuang^{1,†}, Apolline Maitre^{1,2,3}, Ryan O M Rego^{4,5}, Lianet Denis-Abuin^{1,6}, Elianne Piloto-Sardiñas^{1,7}, Natalia Fernández-Ruiz^{8,9}, Jennifer Maye¹⁰, Justė Aželytė¹¹, Pavle Banović^{12,13}, Vaidas Palinauskas¹¹, Dasiel Obregon¹⁴, Alejandro Cabezas-Cruz¹

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† Equal contribution

Abstract

The gut microbiome undergoes natural selection pressure, likely because it can affect infection resistance by stimulating natural antibody (NAb) production, notably against the glycan Gal α 1-3Gal β 1-4GlcNAc-R (α -gal). This study investigates whether specific glycans, such as α -gal, from host microbiota components can trigger NAbs that cross-react with bacterial strains in the *Ixodes ricinus* tick microbiota, potentially altering the tick's ability to transmit *Borrelia afzelii*, a leading cause of Lyme disease in Europe. Oral administration of various *E. coli* strains to mice resulted in the stimulation of NAbs and strain-specific changes in the tick microbiota, significantly decreasing *Borrelia* colonization in the tick vector. Similarly, vaccination with the glycan α -gal induced substantial alterations in the tick microbiota and reduced *Borrelia* colonization. These effects were marked by changes in bacterial diversity, abundance, and microbial network properties. This study provides compelling evidence that natural mechanisms, such as NAb production in response to the gut microbiome, can modulate the microbiota of disease vectors and decrease their competence as carriers of vector-borne pathogens. These findings present novel insights into potential strategies for reducing the transmission of vector-borne diseases by modulating the host gut microbiome.



CUBA - 2024

Symposium Tick microbiota

Monday September 2nd, Room Cetro - Meliá Cohiba

12:30-13:00

Composition and topology of the *Rhipicephalus microplus* microbiota during an infection with *Babesia bovis*.

Iván Corona-Guerrero¹, Rodrigo Morales-García¹, Emmanuel Castañeda-Villafranca¹, Apolline Maitre^{4,5,6}, Consuelo Almazán¹, Alejandro Cabezas-Cruz⁴, Juan Mosqueda^{1,3}.

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Babesia bovis is one of the main causative agents of bovine babesiosis. This apicomplexan parasite induces characteristic pathological alterations in infected erythrocytes resulting in capillary blockage and nervous signs. Constant efforts are carried out by livestock farmers to control the population of the tick vector and reduce babesiosis outbreaks. For this reason, the development of new control strategies is necessary. Tick microbiota consist in a diverse group of symbiotic, commensal, and pathogenic microorganisms. It has been shown that altering the microbiota population prevents the transmission of apicomplexan pathogens. In this work, we've compared the changes in the microbiota of *R. microplus* during an infection with *B. bovis*. Two male calves were infested with 20,000 *R. microplus* larvae, next, one of the calves was infected with *B. bovis*. Infected and uninfected, engorged females were collected and dissected to extract midguts. Dissection was done the same day (0h) or 72 hours after collection. Total DNA was extracted from multiple samples at 0 h and 72 h and sent for sequencing the V4 region of the bacterial 16s gene using the Illumina MiSeq platform. Sequencing data was analyzed using Aldex2 and Qiime2 software to determine the microbial community composition and NetSwan to build co-occurrence networks. Our results indicate that infected ticks have less diverse microbiota than uninfected ticks, also, microbiota in both groups became less diverse at 72 hours post-repletion. Using these data, we described the topology of the co-occurrence networks and determined the keystone taxa of *R. microplus* microbiota. These keystone bacteria can be targeted for remodeling the tick's microbiota, lessening its fecundity and vectorial capacity. This work was funded by the CONAHCYT project number 321162 in collaboration with ANUIES- ECOS NORD (France).



CUBA - 2024

Symposium Tick microbiota

Monday September 2nd, Room Cetro - Meliá Cohiba

13:00-13:30

Differential nested patterns of bacterial pathogens and endosymbionts across *Rhipicephalus microplus* ontogeny.

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Rhipicephalus microplus, or the tropical cattle tick, significantly impacts global livestock. These ticks can transmit *Anaplasma marginale*, the causative agent of bovine anaplasmosis. *Coxiella*-like endosymbionts (CLE) are prevalent within *R. microplus* populations, forming integral components of the tick's microbiome. Research into CLE reveals their potential roles in tick physiology, development, and vector competence. In our study we employ nestedness theory to analyze microbial community dynamics in the *R. microplus* microbiota, focusing on CLE and *A. marginale*. The investigation spans the ticks' ontogeny, tracking CLE and *A. marginale* from larvae to nymphs to adults. We hypothesize that mutualistic CLE will exhibit a nested pattern, reflecting a stable association with the host, while pathogenic *A. marginale* will show a more dynamic pattern influenced by external factors. Our analysis of bacterial community diversity across stages reveals significant variations, with nymphs exhibiting higher diversity. *Coxiella* and *A. marginale* were consistently present, with discernible differences in nested patterns, indicating the secondary nature of *A. marginale* acquisition during feeding, contrasting with the evolutionarily stable relationship with CLE. Core association networks display conserved correlation patterns across stages, but neither *A. marginale* nor CLE shares the same patterns from one stage to another. Overall, our study elucidates the distinct roles of CLE and *A. marginale* in the *R. microplus* microbiota, providing insights into the complex interactions shaping vector microbiota assembly. These findings contribute to understanding the priority effect in community assembly, potentially guiding novel strategies for managing vector-borne diseases in livestock and public health settings.

Symposium Tick microbiota

Monday September 2nd, Room Cetro - Meliá Cohiba

15:00-15:45

***Midichloria* bacteria, unique bacterial symbionts of ticks: from evolution to function**

Davide Sassera

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Midichloria is a genus of intracellular bacteria belonging to the order *Rickettsiales*, living in a symbiotic relationship with multiple tick species. First discovered in *Ixodes ricinus*, these symbionts have been found in many other hard tick species, both within the genus and in the *Metastrata*.

In some of these tick species, *Midichloria* reside not only in the cytoplasm, but also inside mitochondria of the cells of their hosts. Through the years, multiple approaches have been used to unravel the complexities of this interaction, unique among intracellular symbioses.

Through 3D-electron microscopy a detailed characterization of the morphology of the colonized mitochondria was obtained. We discovered that *Midichloria* localize between the organelle's outer and inner membranes, that they modify mitochondrial morphology, but also modulate the tick mitochondrial network, with potential effects on the organelle function.

Comparative genomics allowed to investigate the origin and evolution of the ability of *Midichloria* to colonize mitochondria. This approach identified a set of candidate genes present only in the members of the genus that can colonize the organelles, lost by those that cannot. These results indicate a role of these two apparatuses, possibly through unknown effectors, in regulating the interaction between bacteria and mitochondria.

Currently we leveraged dual-RNAseq results and developed an *in silico* structural biology pipeline for protein-protein interaction predictions, to perform a high-throughput screening testing *Midichloria* proteins against the whole *I. ricinus* mitochondrial proteome. The most promising *Midichloria* protein, potentially capable of modulating mitochondrial activity, is currently being silenced *in vivo*, to investigate its function.

Symposium Tick microbiota

Monday September 2nd, Room Cetro - Meliá Cohiba

15:45-16:15

**Exploring innovative strategies for tick-borne pathogen control through
multi-antigenic peptide vaccination**

Apolline Maitre^{1, 2, 3}, Lourdes Mateos-Hernandez¹, Alejandra Wu-Chuang¹, Foucault-Simonin Angélique¹, Obregón Dasiel⁴, Jean-Christophe Paoli², Alessandra Falchi³, Sara Moutailler¹, Andrea Fogaça⁵, Alejandro Cabezas-Cruz¹

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Borrelia afzelii and *Rickettsia helvetica* are causes of Lyme disease and spotted fever in Europe, respectively. The absence of effective preventive measures against these tick-borne pathogens (TBPs), underscores the need for new strategies. This study investigates whether disrupting the tick microbiota through host antibodies could hinder pathogen acquisition by ticks. Microbiota analysis of ticks collected from cattle (*Rhipicephalus bursa*, *Hyalomma marginatum*) or humans (*Ixodes ricinus*) identified critical bacteria for potential vaccine targets, utilizing a novel Multi-Antigenic Peptide (MAP) approach. *Staphylococcus* emerged as a common bacterium within the microbiota of these tick species. A MAP-based vaccine was developed using a peptide from *Staphylococcus epidermidis* surface protein, with a live *S. epidermidis* vaccine for comparison. We then evaluated the effectiveness of both live and MAP-based *Staphylococcus* vaccines in inducing antibodies that could alter the microbiota of *I. ricinus* nymphs fed on vaccinated mice. Additionally, we investigated if these vaccines could prevent ticks from acquiring *B. afzelii* or *R. helvetica*, utilizing a known *Borrelia* infection model in C3H mice and establishing a new *R. helvetica* infection model in the same mouse strain. Nymphs that fed on mice infected with either bacteria and vaccinated with the MAP-based vaccines exhibited changes in microbiota diversity and composition, indicating the MAP's impact. However, the *Staphylococcus* vaccine did not significantly affect the *Borrelia* spirochete load, contrasting with previous studies using *Escherichia*, underlining the specificity required in microbiota-driven vaccines. Although the *Rickettsia* infection model in mice was successful, the study could not determine the vaccine's effect on this pathogen due to the ticks' low pathogen acquisition rate. This research underscores the innovative potential of MAP vaccines in manipulating tick microbiota to combat TBPs, despite challenges in affecting certain pathogen loads directly. The study demonstrates a promising avenue for controlling these pathogens by targeting the microbial communities within ticks, paving the way for further exploration and development of microbiota-driven vaccines, offering a hopeful strategy for preventing tick-borne diseases.

Symposium Tick microbiota
Monday September 2nd, Room Cetro - Meliá Cohiba

16:15-17:00

Defining Tick Microbiota

Alejandro Cabezas-Cruz

INRAE, France

The study of microbiota within ticks is revolutionizing our understanding of disease transmission and prevention. The microbiota, a complex assembly of microorganisms such as bacteria, fungi, viruses, and protozoans, plays a significant role in influencing a tick's health, disease resistance, and susceptibility to pathogens. Research into tick-microbiota-pathogen interactions has revealed that the microbiota can directly impact the efficiency of disease transmission by ticks, suggesting that manipulating tick microbiota could be a novel strategy for controlling tick-borne diseases. For instance, alterations in the microbiota composition can reduce a tick's reproductive fitness or modify pathogen life cycles, potentially lowering the risk of transmitting diseases like Lyme disease. Addressing the ontological challenges, which involve clarifying the definitions and concepts used in microbiota research, and the epistemological challenges, which concern evaluating the methodologies and theoretical frameworks guiding this research, is crucial. By refining our understanding and methodologies, we can enhance our exploration of the complex interactions within tick microbiota and their implications for disease dynamics. This talk will explore how tick microbiota research is opening up new pathways for preventing vector-borne diseases. As research continues to evolve, it holds the potential to uncover novel disease control strategies, emphasizing the critical role of the microbiota in the dynamics of vector-borne diseases.

Keywords: microbiota, ontology, epistemology



CUBA - 2024

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

9:30-10:00

When bacteria and ticks work together – how dogs deal with Lyme borreliosis

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Due to travelling histories of pets and increasing numbers of imported animals, tick-borne infections become more important in countries of temperate climate zones. Ticks can serve as vectors for different pathogens; assays that detect multiple agents simultaneously is an essential issue.

Canine Lyme borreliosis (LB) is a disease common and well-documented in the northeastern and midwestern parts of the USA, while in Europe the clinical presentation and even the existence of the disease are controversially discussed. Fever and arthritis are clinical manifestations most often associated with LB; other presentations such as renal, cardiac, neurological, muscular disorders are suspected to be sequels of *Borrelia burgdorferi* infection but have not been reproduced experimentally.

Ixodes spp., tick vectors of many tick-borne diseases, might display an increase of their activities and geographical range due to climate change and warmer temperatures. Thus, it is important to consider alternative control methods, possibly by influencing the fitness and pathogen transmission capacity of *Ixodes* spp. by manipulating their endosymbionts. However, comprehensive of predominant bacterial communities is inevitable.

This presentation will show that canine LB may present itself in various clinical forms and should be considered not only in case of musculoskeletal problems or impaired general condition, but also in case knowledge of renal and cardiac disorders. Further research is required to investigate this disease in dogs.



CUBA - 2024

11TH TTP
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Oral presentations

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

10:00-10:30

Transmission of canine filarial worms by ticks

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Dermal filarioids of the genus *Cercopithifilaria* are little studied, yet widespread parasites comprising 28 species living beneath the skin of several vertebrate hosts, including dogs. These parasites have the peculiarity of being the only nematodes transmitted by hard ticks to the definitive hosts. Within this genus, three taxa (i.e., *Cercopithifilaria baina*, *Cercopithifilaria grassii* and a yet undescribed species, namely *Cercopithifilaria* sp. II) infect dogs worldwide, with their occurrence overlapping the distribution of the main tick vector, *Rhipicephalus sanguineus* sensu lato. These dermal filarioids have been associated with clinical signs such as dermatitis, chronic polyarthritis, formation of cutaneous cysts, and possibly influencing infections by other tick-borne pathogens. The biology of *Cercopithifilaria* spp. has received more attention in the last decades following the detection of microfilariae (mfs) of these filarioids in dogs from several regions of the world. The role of brown dog ticks as intermediate hosts/vectors of *C. baina* was assessed recently, in Italy, and since then, larvae stages of *Cercopithifilaria* spp. were detected in *R. sanguineus* s.l. ticks from many countries worldwide. Most studies investigating the biology of these filarioids have been performed for *C. baina* with mfs found to be distributed unevenly in the superficial dermal tissues of infested dogs, being mostly present on the skin of the most common attachment sites of *R. sanguineus* s.l. ticks (e.g., head, ears, and neck regions). Ticks may acquire the infection during all life stages (i.e., larvae, nymphs, and adults) by ingesting mfs, which subsequently develop into L1 (in about 10 days), L2 (~20 days), and L3 (~30 days), within the tick gut. Therefore, transstadial passage of the infection has been demonstrated in brown dog ticks, with infected nymphs becoming infected adults. Finally, L3s are transmitted to dogs by infected *R. sanguineus* s.l. nymphs or adult ticks, and in the definitive host these larvae will undergo further development until they become adults, which are usually located in the subcutaneous tissues.



CUBA - 2024

11TH TTP
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Oral presentations

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

10:30-11:00

Canine Monocytic Ehrlichiosis - An Update

Shimon Harrus, DVM, PhD, Dipl. ECVCP

Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel.

Canine monocytic ehrlichiosis (CME), caused by the obligate intracellular bacterium *Ehrlichia canis* is an important disease of dogs and other canids worldwide. It is transmitted by ticks of the *Rhipicephalus sanguineus* complex. Common clinical signs of CME include depression, lethargy, anorexia, weight loss, lymphadenomegaly, splenomegaly and bleeding, mainly subcutaneous and mucosal petechiae and ecchymoses. The most common hematological sign of CME is thrombocytopenia occurring in more than 90% of infected dogs. A negative relationship was found between the degree of thrombocytopenia and the presence of *E. canis* DNA in the dogs' blood, in endemic regions. Dogs may also develop mild non-regenerative anemia and leukopenia following infection. Pancytopenia is a common hematological finding in dogs suffering from the chronic severe form of the disease.

The suspicion of CME should be considered when a compatible history (tick infestation, travel to or living in endemic region), typical clinical signs, typical hematological and biochemical abnormalities (hypoalbuminaemia, hyperglobulinaemia) are present. Classical diagnostic techniques (hematology, cytology, serology, isolation) are useful tools in the diagnosis of CME. Demonstration of a typical morula within the cytoplasm of a monocyte in blood smear indicates a monocytotropic ehrlichiosis. In rare cases, *E. canis*-morulae may be seen in lymphocytes, neutrophils and eosinophils. Blood smears of infected dogs may present reactive monocytes, erythrophagocytosis, platelet phagocytosis and granular lymphocytosis. The indirect immunofluorescence antibody (IFA) test is considered the serological "gold-standard" test, indicating exposure to *E. canis*. It is considered as a valuable screening test for CME. IgG titers of 1:40- 1:80 or greater are considered positive. Two consecutive IFA tests, 7-14 days apart, are recommended, and a 4-fold increase in antibody titers is suggestive of an active infection. Point of care enzyme linked immunosorbent assay (ELISA) kits for the detection of *E. canis* are also available (e.g. Snap 4Dx® assay by IDEXX Laboratories Inc., USA and the Immunocomb® by Biogal, Israel). They are sensitive and specific and are in common use in clinics. A definitive diagnosis of *E. canis* infection should be done by polymerase chain reaction (PCR) and sequencing. Polymerase chain reaction and sequencing are sensitive methods for detecting and characterizing *E. canis*, respectively. Common PCR targets are the 16S rRNA, p28, p30, disulfide oxidoreductase, gp19, gp36 and the gp140 gen loci. Detection of *E. canis* DNA can be achieved as early as 4 to 10 days post-inoculation.

Tetracyclines in general and doxycycline in particular are the therapeutic agents of choice for the treatment of CME. Doxycycline should be administered at a dose of 5mg/kg q12 hrs or 10mg/kg q24 hrs for the duration of 3-4 weeks for dogs at the acute phase. Dogs in the subclinical phase may require prolonged treatment. The prognosis of the acute and the subclinical phases of the disease is good, however grave for the chronic phase. Dogs in the chronic phase will eventually die due to bone marrow hypoplasia and its outcomes: peripheral pancytopenia, sepsis and/or bleeding. To date, there is no commercial vaccine and tick control is the most effective preventive measure.

Molecular and serological reports from Brazil, Portugal, Angola, St. Kitts, West Indies and Qatar suggest that a new genotype/variant of *E. canis* infects cats. However, attempts to isolate *E. canis* from cats was unrewarding to date. *Ehrlichia canis*-DNA was detected in humans from Venezuela, Costa Rica and Panama suggesting that a closely related organism or strain may infect humans in South and Central America.



CUBA - 2024

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

11:30-12:00

Ticks and tick-borne pathogens of carnivores in Europe: the wild-domestic interface

Mihalca Andrei Daniel

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The vast majority of pathogens, including parasites, are shared between wild and domestic canids and felids worldwide. This is related mainly to close evolutionary relationship of these hosts but also to shared ecological and biological features such as habitat sharing, behaviour or diet. Hence, the spillover of pathogens from wildlife to domestic hosts, including zoonotic spillover is not uncommon. Such events have recently witnessed an emerging trend, due to various factors such as changes in wild or domestic host demographics or environmental changes, including wildlife urbanization as well as increased interactions due to social changes on the domestic side. Tick-borne pathogens are not an exception. The role of wild carnivores in the ecoepidemiology of tick-borne diseases in Europe is only partly understood, as the reservoir role of these hosts has been poorly investigated. Moreover, wild carnivores seem to harbour a great and incompletely known diversity of tick-borne pathogens, including ones which can pose health threats to pets or humans. New species of tick-borne apicomplexans have been recently described in a variety of wild carnivore hosts in Europe and there is an increasing series of evidence that these hosts can play an important role as reservoirs for zoonotic tick-borne bacteria. However, the amount of knowledge gaps is even more pronounced. All these aspects are to be discussed in detail during the presentation.

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

12:00-12:30

Pathogenesis and treatment of canine babesiosis

Gad Baneth

The Koret School of Veterinary Medicine, The Hebrew University, Rehovot, Israel

Canine babesiosis caused by different *Babesia* species is a protozoal tick-borne disease with global importance and worldwide distribution. *Babesia* species infecting dogs were historically identified based on the morphologic appearance of the parasite in the erythrocyte. All large forms of *Babesia* were designated *Babesia canis* and all small forms of *Babesia* were considered to be *Babesia gibsoni*. But, the development of molecular methods and the study of tick vector-competence as well as pathogen proteomics and host immune responses have demonstrated that additional *Babesia* species infect dogs and cause distinct diseases. The geographical distribution of canine *Babesia* species and thus the occurrence of babesiosis are largely dependent on the habitat of relevant tick vector species, with the exception of *B. gibsoni* where evidence for dog to dog transmission indicates that infection can be transmitted among fighting dogs breeds independently of transmission by ticks. Knowledge of the geographic distribution and clinicopathological changes in dogs infected by different *Babesia* species is of epidemiological and medical importance. *Babesia* infection causes a disease with clinical manifestations that may vary considerably with the different species and strains involved and also with factors that determine the host's response to infection such as age, individual immune status, and the presence of concurrent infections or other diseases. Hemolytic anemia is the hallmark of this disease. When it is associated with systemic inflammatory responses it may lead to tissue hypoxia and organ dysfunction which account for the clinical signs observed in severe canine babesiosis. Babesiosis caused by large *Babesia* species is treated with imidocarb dipropionate or diminazene aceturate while small *Babesia* species are more resistant to these drugs and require treatment with combinations of other drugs such as atovaquone, azithromycin and clindamycin. Accurate detection of infection and species recognition are important for the selection of the correct therapy and predicting the course of disease. Resistance to some drugs such as atovaquone has been described and its mechanism has been studied.



CUBA - 2024

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

12:30-13:00

Tick-borne diseases in dogs and cats in Latin America

Filipe Dantas Torres

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Latin America is a region of the Americas where Spanish and Portuguese languages are predominantly spoken. In this definition, the Guianas (French Guiana, Guyana, and Suriname), the Anglophone Caribbean (and Belize), the Francophone Caribbean and the Dutch Caribbean are often excluded from this region. Geographically speaking in a broader sense, Latin America includes Mexico, Central America, the Caribbean, and South America. This region encompasses an area of over 20 million square kilometres, with a great diversity of biomes and climate types. Consequently, the region is home to an outstanding variety of animals, including invertebrates that may act as vectors of disease agents. This is the case of ticks, which are widespread and abundant in Latin America. In fact, animals and humans living in this region are at risk of several vector-borne diseases, including tick-borne diseases such as anaplasmosis, babesiosis, ehrlichiosis, hepatozoonosis, rangeliiosis, and rickettsiosis. Cases of tick toxicosis (a non-infectious syndrome due to tick bite) have also been reported in dogs and humans. In this talk delivered in the special symposium *Tick-borne diseases of dogs and cats*, I review the main tick-borne diseases of dogs and cats in Latin America.

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

13:00-13:30

TICK BORNE DISEASES OF DOGS AND CATS IN INDIA

Sangaran Arumugam and Munagala Sreevidhya

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Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.

India's diverse climatic zones, ranging from montane and semi-arid regions to wet tropics, provide suitable habitats for a wide array of vectors and pathogens of medical and veterinary significance. The increased occurrence of companion animal vector-borne diseases in India, more so by ticks is impacted by a blend of environmental and social elements. The transmission and geographical distribution of these vectors and pathogens are intricately linked to regional variations in temperature, rainfall, and humidity. CVBD's are widespread across India, yet precise identification of the specific species involved remains largely anecdotal. Frequently encountered CVBDs in India through ticks include hepatozoonosis, ehrlichiosis, babesiosis, mycoplasmosis and cytauxzoonosis. Specifically for cats having access to the outdoors are more vulnerable to these infections. Ticks play an important role in the transmission of babesiosis, hepatozoonosis and cytauxzoonosis. In India, CVBD's are highly prevalent, presenting significant challenges to pet practitioners due to their clinical complexities, especially in terms of diagnosis and treatment. Many laboratories use conventional parasitological techniques, which have limited sensitivity and specificity. Due to resistance observed in multiple drugs, it's crucial to explore novel drug options and examine the combined effects of drug combinations. The increase in drug resistance among vector and pathogen populations could also contribute to the spread of CVBDs especially through tick transmission.



CUBA - 2024

11TH TTP
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Oral presentations

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

15:00-15:30

Anaplasmosis of dogs and cats

Smaragda Sotiraki

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The genus *Anaplasma* (Rickettsiales: Anaplasmataceae) comprises obligate intracellular Gram-negative bacteria transmitted by Ixodid ticks, causing infections of wild/domestic animals worldwide with a proven or potential zoonotic role that have shown increasing prevalence worldwide. Canine anaplasmosis is caused by *A. phagocytophilum* which infects peripheral neutrophils and eosinophils and causes canine granulocytic anaplasmosis and *A. platys* which is a platelet-specific pathogen causes canine infectious cyclic thrombocytopenia. *A. phagocytophilum* is transmitted by different *Ixodes* species, based on geography (e.g. *I. ricinus* in Europe and *I. scapularis* and *I. pacificus* in Northern America) however the bacterium has been detected in several other hard tick species with no clear epidemiological role. The natural mode of transmission of *A. platys* has not been demonstrated conclusively, but so far it seems that *Rhipicephalus sanguineus* is the main vector.

Natural Infections of cats with *Anaplasma* species are less frequent than in dogs and in most cases exposure to those pathogens is reported only based on DNA or antibodies detection. Nevertheless, there are several feline clinical cases showing lethargy, loss of appetite, fever, severe inflammatory processes, and thrombocytopenia Worldwide, the most frequently reported species are *A. phagocytophilum*, and more rarely *A. platys*. The same vectors responsible for infections in dogs are suspected to transmit them to cats.

Anaplasma infections are challenging to diagnose presenting with nonspecific clinical signs that differ depending on the agent involved, the affected host, and other factors such as immune status and coinfections. Because every assay has strengths and limitations, a negative result from cytology, serology, or PCR does not necessarily rule out infection. The combination of multiple diagnostic tools is recommended to optimize the diagnosis across the different stages of infection. Doxycycline or minocycline are the drugs of choice and therapy scheme should be based on clinical manifestation and specific diagnostic testing results.

Infection in dogs and cats suggests the presence of the vector in the same environment to which humans may also be exposed. Therefore, vector control is the most effective prophylactic measure, not only to protect animals' health but also to avoid the development of reservoirs for humans.



CUBA - 2024

11TH TTP
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Oral presentations

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

15:30-16:00

Canine and feline hepatozoonosis – coevolution of parasites with their hosts

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Hepatozoon is a genus of apicomplexan parasites transmitted to vertebrate hosts by hematophagous arthropods like ticks and mosquitoes. *Hepatozoon canis* and *Hepatozoon felis* are transmitted to dogs and cats, respectively, by the ingestion of ticks carrying sexual stages of the parasites. Infections may lead to non-specific signs like fever, weight loss, lymphadenopathy, and lethargy or even course with anemia, leukocytosis and other life-threatening manifestations in puppies, or immunocompromised animals. In other vertebrate species, *Hepatozoon* spp. infections usually undergo without clinical manifestations, suggesting a long coevolutionary history between hosts and parasites. Interestingly, these parasites tend to infect specific host groups, for instance *H. canis* parasitizes different canid species, *Hepatozoon ursi* usually infects bears or *H. felis* infects domestic cats and other felids.

The global coevolution and possible coevolutionary events between *Hepatozoon* spp. and their vertebrate and invertebrate hosts were evaluated by using phenetic and patristic distances and the most parsimonious reconciliations based on their phylogenies. Results showed cophylogenetic congruence between *Hepatozoon* spp. and species of the order Carnivora, Rodentia and Squamata with PACo $m^2_{XY} < 3.17$ and $p < 0.00001$. In addition, the event-based method showed that this congruence was mainly explained by host switch ($p < 0.01$ for all cases). On the contrary, no overall coevolutionary congruence was found between *Hepatozoon* spp. and their tick hosts, although significant links between *H. canis* and *Rhipicephalus* spp. were observed.

This suggests that *Hepatozoon* spp. infect new vertebrate hosts possibly due to sympatry of permissive and phylogenetically related hosts, whereas cospeciation does not seem to be the major coevolutionary force in this intricate host-parasite association. Altogether, the changing dynamics of *Hepatozoon* infection in ticks and vertebrates may lead to the emergence of new infected hosts, thus, awareness of different *Hepatozoon* spp. truly infecting domestic animals should be raised.



CUBA - 2024

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

16:00-16:30

***Midichloria mitochondrii* stimulates the sylvatic cycle of Lyme spirochetes in *Ixodes ricinus* instars**

Clara F. Köhler¹, Maya L. Holding², Manoj Fonville¹, Ron P. Dirks³, Hans J. Jansen³, Sara Moutailler⁴, Aurélie Heckmann⁴, Jens Zarka⁵, Erik Matthysen⁵, Aleksandra I. Krawczyk⁶, Hein Sprong¹

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5. Evolutionary Ecology, Department of Biology, University of Antwerp, Wilrijk, Belgium
6. Mitrani Department of Desert Ecology, The Swiss Institute for Dryland Environmental and Energy Research, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben-Gurion, Israel

Ixodes ricinus nymphs carrying the vertically transmitted *Midichloria mitochondrii* (*Midichloria*) are more often infected with the horizontally transmitted *Borrelia burgdorferi sensu lato* (*Borrelia*), and female nymphs take a larger bloodmeal than males. Also, female adult ticks have more often *Midichloria* than males. Here, we aimed to disentangle the role of the sex and *Midichloria* presence in tick instars in the sylvatic cycle of *Borrelia*. For this, we developed a qPCR for the specific detection of male *I. ricinus*.

There was no difference in the infection rate of *Midichloria* between male and female larvae, and neither its presence nor the sex affected the size of larvae. However, the infection with *Midichloria*, not the sex, increased the uptake of *Borrelia* in larvae feeding on *Borrelia*-infected birds. This aligns with the finding that the infection with *Borrelia* in questing nymphs was positively associated with *Midichloria*, but not with their sex. Remarkably, female nymphs were more (often) infected with *Midichloria* than males. Both the presence of *Midichloria* and the (female) sex were positively associated with the size and weight of nymphs. We further corroborate that the infection rates of *Midichloria* in ticks shows substantial spatial variation, and now show that locations with a higher *Midichloria* prevalence in nymphs have a significantly higher infection rate with *Borrelia*.

Thus, *Midichloria* alters the propensity of *I. ricinus* instars to facilitate the sylvatic cycle of *Borrelia*, and is therefore an additional factor that contributes to the spatial variation in Lyme disease risk.

Symposium Tick-borne diseases of dogs and cats

Monday September 2nd, Room La Corona - Meliá Cohiba

16:30-17:00

The first report of *Hepatozoon felis* in a domestic cat in Moscow (Russia)

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Currently, three species affecting wild and domestic European cats have recently been described and defined as *Hepatozoon felis*, *Hepatozoon canis* and *Hepatozoon silvestris*. *Hepatozoon canis* infection is widespread and have been reported in Russia from dogs. DNA *Hepatozoon felis* was detected in tick collected from Amur tigers in Russian Far East. However, until this moment there were no published cases of hepatozoonosis in domestic cats. Herein, we describe the case of detection of *H. felis* in abdominal effusion of a cat.

A Siberian male cat, 7,5 y.o., was presented to the veterinary clinic for ultrasound examination. Ultrasound revealed increased echogenicity of the renal parenchyma, increased echogenicity of omentum and enlargement of the mesenteric lymph nodes. Also, moderate amount of effusion was found in the abdominal cavity. Fluid was obtained for microscopic examination and PCR for FCoV. Cytology of abdominal fluid revealed aseptic neutrophilic inflammation with presence of moderate number of macrophages and lymphocytes and small number of eosinophils. Hamonts of *Hepatozoon* were observed in some neutrophils. Partial sequencing of 18S rRNA gene confirmed *Hepatozoon felis* infection. Furthermore, effusion sample showed positive result in the PCR for feline coronavirus. To our knowledge, this is the first case of detection of *Hepatozoon felis* in a domestic cat in Russia and concurrent disease for feline infectious peritonitis.



CUBA - 2024

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

9:30-10:15

Water management of *Ixodes ricinus* and *Dermacentor marginatus*, two hard tick species in Central Europe with different life cycle strategies

Olaf Kahl

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Ixodes ricinus and *Dermacentor marginatus* are two hard tick species with an overlapping distribution in parts of Europe but with differing microhabitat preferences. The former occurs in and near forests or forest-like habitats (parks, cemeteries, neglected gardens etc.). With the exception of phases when questing, all the other developmental off-host phases (engorged, moulting, resting unfed) are spent in the leaf litter, which offers a rather humid micro-environment in all seasons. In contrast, *Dermacentor marginatus* prefers steppe-like, more open landscapes (dry grassland) with a mix of trees, bushes and grassy terrain. The adult stage tolerates periods of low relative humidity much better than *I. ricinus*. In the present study, the water management of the postembryonic stages of both species was investigated. This included the capability of active water vapour uptake and the significance of metabolic water for their water balance. Tick body mass (fresh mass, dry mass, water content) was determined by weighing them intermittently on a microbalance or continuously on a recording ultramicrobalance. For the interpretation of the findings, it is essential to consider the seasonal timing of the life cycles of both species, especially their longevity between the bloodmeals and their capability of entering a developmental diapause. The immatures of *D. marginatus* are obligatorily short-lived, whereas *I. ricinus* immatures are long-lived and can enter a developmental diapause in the engorged state. The development from oviposition to the F1 adult stage of *I. ricinus* needs several years in Central Europe, whereas the same developmental steps in *D. marginatus* have to be completed within only one vegetation period. The unfed adults are long-lived in both species. These different life cycle strategies are clearly reflected in differences in their water management and in their net intake of water versus dry matter during the bloodmeals.



CUBA - 2024

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

10:15-10:45

**These legs are made for sensing:
Carbon dioxide enhances *Ixodes* responsiveness to tactile cues**

Carola Städele

Institute for Neuro- and Sensory Physiology, University of Göttingen Medical Center; Göttingen, Germany

Carbon dioxide (CO₂) is considered a crucial behavioral activator and attractant for ticks and an essential sensory cue in host-seeking. However, the exact mechanisms behind how CO₂ activates and facilitates host-seeking in ticks are not well understood yet. Therefore, we conducted a study to investigate CO₂ responses in *Ixodes scapularis* (North America) and *Ixodes ricinus* (Europe).

In our study, adult ticks were exposed to different concentrations of CO₂ (1%, 2%, 4%, and 8%), and we analyzed changes in their walking behavior and foreleg movement. We found that CO₂, even at lower concentrations (1%), is a potent stimulus for both *Ixodes* species. The behavioral responses of the ticks depended on their state: walking ticks increased their walking speed, while stationary ticks began waving their forelegs and displaying questing behavior, both of which are aspects of host-seeking.

Interestingly, both *Ixodes* species showed no clear preference for any specific concentration of CO₂, and they were not more sensitive to breath like CO₂ concentrations (~4%) than to other tested concentrations. This suggests that the animals respond once the CO₂ level exceeds a certain threshold. Furthermore, CO₂ is a behavioral activator that enhances *Ixodes* responsiveness to tactile stimuli. After exposure to CO₂, both *Ixodes* species were more likely to attach to a passing object than without prior CO₂ exposure.

Moreover, we found that the foreleg Haller's organ is likely involved in CO₂ detection, but it is not necessary. Even with the Haller's organ covered or amputated, both *Ixodes* species strongly responded to CO₂. This implies that there must be CO₂-sensitive structures important for host-seeking in ticks that still need to be identified.

Symposium Tick Physiology and Metabolism
Tuesday September 3th, Room Cetro, Meliá Cohiba

10:45-11:00

Unraveling Tick Lipid Metabolism: 'Omics' and Biochemistry Perspectives

Tereza Kozelková^{1,2}, Filip Dyčka^{1,2}, Matthias Schittmayer-Schantl³, Veronika Urbanová¹, Štěpán Strnad⁴, Vladimír Vrkoslav⁴, Ruth Birner-Gruenberger³, and Petr Kopáček^{1*}

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Tick physiology, development and reproduction depend entirely on the ingestion and processing of host blood as their primary nutrient source. In contrast to the relatively well-studied digestion of proteins, almost nothing is known about the uptake and utilization of host lipids. Several genes encoding lipolytic enzymes have been identified in the midgut transcriptome of the hard tick *Ixodes ricinus*. However, classical proteomics failed in identifying corresponding proteins due to the presence of other highly abundant proteins.

This comprehensive work offers the first in-depth study of poorly understood lipid metabolism in ticks. Various 'omics' and biochemical tools were used to characterize lipolytic enzymes, their activity, and midgut lipid composition, alongside lipid droplets dynamics were observed using microscopy techniques.

Another challenge for the tick parasitic lifestyle is the long starvation period outside the host. Ticks must survive from energy resources (mainly in the form of lipids) obtained during their previous life stage. Lipid droplets in the midgut during nymphal starvation were observed using the fluorescent dye LD540, revealing the rapid formation of lipid droplets in the early phase of blood intake and the utilization of lipids during the long fasting periods.

The proteomics data were extended by assessing total lipase activity (TLA) in tick midgut homogenates from different feeding timepoints using a universal fluorescent substrate (4-Methylumbelliferyl oleate). TLA was found to increase until 5th day of feeding and then rapidly decreased. Commercially available lipase inhibitors were investigated to characterize the lipolytic system. In addition, a lipidomic analysis was performed to identify the lipid classes in the unfed and partially fed ticks.

To further identify proteins responsible for the lipolytic machinery, activity-based-proteomics was carried out by using a C6-activity-based probe and Label-free quantitative (LFQ) proteomics. Several hydrolases from different classes were detected, active either in unfed ticks and/or during feeding stages.

Acknowledgments: Supported by GACR 21-08826S and GAJU 054/2022/P



CUBA - 2024

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

11:30-12:15

Functional properties of *Ixodes ricinus* cholinergic receptors expressed in the synganglion

Steeve Thany

Université d'Orléans, France

Ticks are known to transmit the largest number of pathogens among arthropod vector seriously affecting both human and animal health. The European castor-bean tick *Ixodes ricinus* is the primary vector of *Borrelia* spirochetes causing Lyme disease and is also well-known vector of tick-borne encephalitis virus. Among, distinct neutral mechanism that have been described in control of tick salivary gland, the most enigmatic remains to be the cholinomimetic-mediated salivary gland fluid secretion. Specifically, it has been speculated that this action is an indirect process, likely linked to an activation of the putative muscarinic-acetylcholine receptors in tick synganglion. In the tick *Ixodes ricinus*, the functional properties of cholinergic receptors located in the synganglion neurons are still unknown. We first characterized the pharmacological properties of tick nicotinic and muscarinic receptors using synganglion membranes microtransplantation in *Xenopus laevis* oocytes and two electrode voltage clamp method. We found that oocytes microtransplanted expressed nicotinic and muscarinic receptor subtypes. Then, we isolated for the first time single neurons from *I. ricinus* synganglion and use a patch-clamp method in whole cell configuration to investigate pharmacological action of cholinergic drugs on these cells. We identify different cell types by there size, the pharmacological properties and calcium imaging.

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

12:15-12:55

Activities of Two Types of Axonal Muscarinic Acetylcholine Receptors Mediate Formation of Saliva Cocktail in the Tick *Ixodes ricinus*

Caina Ning¹, James J. Valdés², Lourdes Mateos-Hernandéz¹, Sabine Rakotobe¹, Lianet Abuin-Denis^{1,3}, Nadia Haddad¹, Livia Šofranková^{1,4}, Khalid Boussaine⁵, Helena Frantová², Veronika Urbanová², Tereza Kozelková^{2,6}, Filip Dyčka^{2,6}, Petr Kopáček², Ondřej Hajdušek², Radek Šíma², Jiří Týč², Tomáš Bílý^{2,6}, Marie Vancová^{2,6}, Jan Perner², Steeve Thany⁵ and Ladislav Šimo¹

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The secretion of salivary gland (SG) in ticks is effectively triggered by the muscarinic acetylcholine receptor (mAChR) agonist, the pilocarpine, known as a worldwide-preferred tool for obtaining copious saliva. However, the physiological significance of this chemical agent is poorly understood. Presently, all we know is, that injection or apical application of pilocarpine, into/on partially-fed tick females induces robust long-lasting salivary secretion, whereas it fails to induce salivation from isolated SG. In addition, cholinomimetics - mediated SG secretion can be effectively abolished by muscarinic antagonist atropine or by cutting neuronal connections between tick synganglion and SG, jointly supporting the involvement of a neural mAChR subtype(s) in these processes. Based on these observations, it has been generally believed that tick synganglion, senses muscarinic agents and subsequently mediates SG fluid secretion via unidentified non-cholinergic "secreto-motor nerves", connecting neuronal cells with SG acini. Currently, the only known processes, associating specific tick central neurons with SG are the neuropeptidergic axons arising from two different sets of neurons and innervating either type II or both type II/III saliva-producing acini. Our results indicate, that in *Ixodes ricinus* both sets of these neuropeptidergic cells are sensitive to muscarinic agents via the expression of pharmacologically distinct type (-A and -B) of mAChR. Furthermore, *I. ricinus* synganglion sensitivity to cholinomimetics is suggested by a rich axonal cholinceptive arborizations at its surface, originating from two prominent clusters of neurosecretory cells expressing either type A or B of mAChR. Following this model, we tested the effect of *in vivo*-injected selective muscarinic agents, to investigate their effect on volume and amount and composition of proteins of *Ixodes* saliva. Finally, we propose that in *Ixodes* females, the co-joint actions of two different neuronal mAChRs mediate a tandem cooperation of type II and III SG acini for saliva cocktail formation.

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

12:55-13:30

The pleiotropic action of a tick salivary serpin on vertebrate haemostasis and its effect on psoriasis-like skin inflammation

Mohamed Amine Jmel¹, Constance C. F. M. J. Baaten^{2,3}, Huimei Wu⁴, Kanin Wichapong², Tilman Hackeng², Xueqing Xu⁴, Kutty Selva Nandakumar^{4,5}, Elisabetta Castoldi², Ingrid Dijkgraaf^{#2}, Michalis Kotsyfakis^{#6}

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#: These authors contributed equally

Abstract

As several physiological processes such as coagulation, immune response and inflammation are driven by proteolysis, protease inhibitors identified in tick saliva have attracted research attention. The identification and functional characterization of exogenous molecules that interfere with physiological pathways in the vertebrate host may provide the basis for developing new biotechnological tools for therapeutics targeting inflammatory and autoimmune pathologies.

Here we present the effects of Iripin-3, a tick salivary serpin, on coagulation, platelet activation and skin inflammation. Iripin-3 inhibited thrombin generation initiated through the extrinsic or the intrinsic pathway of coagulation in a dose-dependent manner. This action was explained by the inhibition of FXIIa, FXIa and thrombin

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

15:00-15:45

Proteolytic enzymes associated with tick gut tissue

David Hartmann^{1,2}, Jana Pytelková³, Katarina Orsághová^{3,4}, Mária Beňová^{3,4}, Nikola Chmúrčiaková³, Pavel Talacko³, Tereza Kozelková^{1,2}, Zdeněk Franta², Radek Šíma¹, Jan Perner¹, Pavla Bartošová-Sojková¹, Veronika Urbanová^{1,5}, Petr Kopáček¹, Michael Mareš³, Martin Horn³ and Daniel Sojka^{1,5*}

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Abstract:

The gut of ticks plays a pivotal role in their ability to digest host blood, a process central to their survival, reproduction, and capacity as disease vectors. This digestive process involves a complex network of mainly cysteine and aspartic proteases breaking down proteins into peptides and amino acids intracellularly, within the endolysosomal system of gut digestive cells. Despite its critical importance, our understanding of tick gut proteolysis remains rather incomplete, with significant gaps in knowledge concerning the diversity of proteolytic enzymes involved, their regulation, and interactions with tick-borne pathogens. This research critically evaluates current knowledge on tick gut proteolysis, focusing on *Ixodes ricinus*, the European vector of Lyme disease. It highlights our approach to identifying predominant digestive proteolytic enzymes through comprehensive "omic" analyses and dynamic expression profiling across tick developmental stages and tissues. Our findings reveal significant differences in tick digestion phases, particularly spotlighting newly identified cathepsin variants, as opposed to the previously characterized forms. These cathepsin isoenzymes play crucial roles in post-rapid engorgement blood storage by their direct role in preventing the clotting of imbibed vast amounts of host blood and its preservation via the production of hemoglobin-derived antimicrobial peptides. This contribution further provides insights into the complexity of tick digestion and gut-related physiology potentially involving also serine and metalloproteases, and its implications for disease transmission. Not least, it presents the concept of selective inhibition of tick proteasome as a strategy to combat ticks. We propose future research directions aimed at identifying and characterizing newly identified proteolytic enzymes, understanding their regulation, and exploring potential targeting of gut proteolysis in tick control strategies. This work not only advances our knowledge of tick biology but also contributes to the development of innovative methods to control tick populations and mitigate their role in spreading diseases."

Acknowledgements: This work was supported by the grants 21-08826S and 24-10659S from the Czech Science Foundation (GAČR)



CUBA - 2024

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

15:45-16:10

Inhibition of 4-Hydroxyphenylpyruvate Dioxygenase (HPPD) Leads to Melanogenic Self-Catastrophe in Ticks

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Ticks imbibe a vast amount of host proteins in their blood meal. Digestion of host proteins occurs intracellularly within tick midgut digest cells. Released amino acids serve as precursors of tick proteosynthesis, neurotransmitters or polyamines synthesis. Excessive amino acids are catabolised to prevent an intracellular amino acid build-up. 4-Hydroxyphenylpyruvate dioxygenase (HPPD) is a key enzyme that catalyses the second reaction in the catabolism of tyrosine. Silencing its mRNA transcripts by RNAi or inhibiting its enzymatic activity via small molecule inhibitors administered through artificial membrane feeding clearly shows that HPPD is an essential enzyme securing post-blood-feeding tick survival. Both approaches confer systemic hyper-tyrosinaemia after blood-meal with tyrosine and phenylalanine being elevated in tissues and haemolymph of ticks. To examine the cause-effect relationship, we have performed a comprehensive untargeted metabolomic analysis of haemolymph of HPPD-inhibited ticks. Clearly, tyrosine flux re-routes towards DOPA formation, which then feeds into melaninogenic and sclerotising pathway. Indeed, using mechanistic studies, the cuticle of HPPD-inhibited ticks loses its plasticity and increases its fragility. We argue that HPPD functioning is a key for tick blood-feeding biology success and that its inhibition interferes with cuticle plasticity, an essential attribute of tick feeding performance and post-feeding survival.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

16:10-16:30

The function of the fat body of *Ixodes ricinus* in tick immunity

Veronika Urbanova^a, Stephen Lu^b, Larissa Martins^c, Eliska Kalinova^a,
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Ticks are blood-feeding parasites that transmit various pathogens. The transmission of pathogens depends on their ability to evade or inhibit the immune response of ticks. Immune molecules, mainly synthesized by the fat body and hemocytes, play a crucial role in tick immunity. While the immune response of hemocytes has been extensively characterized, the role of the fat body in tick physiology and immunity remains largely unexplored.

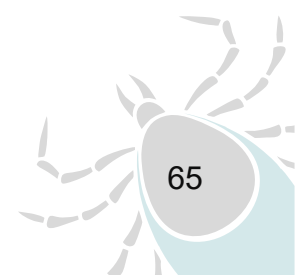
Recently, we performed a transcriptomic analysis of the fat body/trachea complex of partially fed *Ixodes ricinus* females and obtained an overview of the transcripts encoding proteins involved in tick immunity. We evaluated the immune response of the most abundant transcripts following challenges with model microbes at different intervals. Most transcripts showed upregulation in response to microbe injection, with the most significant immune response observed 24 hours post-injection. However, immune transcripts were relatively poorly represented compared to other functional groups, possibly due to low baseline expression levels in the absence of immunologic challenge.

Therefore, we performed immune stimulation followed by transcriptome study. We activated the Toll immune pathway, which is responsible for controlling antimicrobial activity, by RNA interference-mediated silencing of the negative regulator Cactus. We also examined the list of genes that were upregulated following stimulation of the Toll immune pathway compared to the control. The expression of these selected genes was validated by microbial challenge and RNA interference, confirming their role in the Toll immune pathway.

Our data demonstrate the importance of the fat body in ticks as a crucial tissue that makes a remarkable contribution to immune defense against invading microbes. These findings promise a better understanding of immune processes and their regulation in ticks.

Acknowledgment:

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CUBA - 2024

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

16:30-16:45

An inhibitor of apoptosis protein is essential to prevent the death of *Amblyomma sculptum* ticks upon a redox imbalance

Marcelly Nassar^{1*}, Larissa A. Martins², Josiane Betim de Assis³, Eliane Esteves^{1§}, Anderson Sá-Nunes³, Marcelo B. Labruna⁴, Sirlei Daffre¹, Andrea C. Fogaça¹

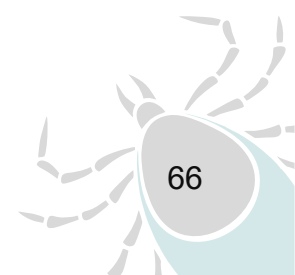
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Rickettsia rickettsii is a tick-borne intracellular bacterium that causes the life-threatening Rocky Mountain spotted fever. In Brazil, where the disease is known as Brazilian spotted fever, the tick *Amblyomma sculptum* is the major vector. After the bite of an infected tick, the bacterium invades and proliferates into the host endothelial cells, causing vasculitis. However, *R. rickettsii* exerts an inhibitory effect on the apoptosis of both the host endothelial cells and the tick vector cells, favoring infection. The inhibitors of apoptosis proteins (IAPs) play a central role in the regulation of apoptosis, directly inhibiting caspase activity. Therefore, an IAP coding sequence (CDS Acaj-73060) of *A. sculptum* was silenced and ticks were fed on rabbits infected or not with *R. rickettsii*. A high mortality of IAP-silenced ticks was observed after blood feeding, independently on the presence of the bacterium. The expression of apoptotic regulators is downregulated in fed ticks, suggesting that the acquisition of the blood meal activates apoptosis, causing the death of IAP-silenced ticks. It is known that the heme released by digestion of the blood meal, among other factors, may induce the production of reactive oxygen species (ROS), activating apoptosis. Therefore, we analyzed the effects of anti- and pro-oxidants on the survival of IAP-silenced ticks. IAP-silenced ticks treated with tocopherol (antioxidant) presented a lower mortality rate (28%) in relation to the control (84%) upon feeding. On the other hand, the treatment with paraquat (prooxidant) caused a high mortality in IAP-silenced ticks (80%) in relation to the control (32%). Together, our results suggest that a redox imbalance activates the apoptosis in the tick midgut, causing the death of IAP-silenced ticks. The essential role played by IAP upon tick feeding highlights this molecule as target for the development of tick control strategies.

Preferred presentation: poster

(Supported by FAPESP 2023/03905-6 and CNPq)





CUBA - 2024

11TH TTP
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Oral presentations

Symposium Tick Physiology and Metabolism

Tuesday September 3th, Room Cetro, Meliá Cohiba

16:45-17:00

Electromagnetic radiation alters the mRNA levels of neuropeptide genes in the *Ixodes ricinus* synganglion

Lívia Šofranková ^{1,2}, Natália Pipová ¹, Miroslav Bañas ¹, Igor Majláth ¹, Juraj Kurimský ³, Roman Cimbala ³, Ladislav Šimo ², Viktória Majláthová ^{1*}

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One of the important environmental factors affecting the functionality of living systems is the omnipresent anthropogenic electromagnetic radiation (EMR). It has been proven in numerous publications that some man-made frequencies can affect cell's electrochemical equilibrium, gene expression levels and proteosynthesis. Several behavioral studies confirm that ixodid ticks perceive EMRs, however, the research of its effects on physiology and the molecular aspects of tick's reactions is neglected. Presented study is focusing on the effects of EMR on the neuropeptide expression levels in the synganglion of the sheep tick (*Ixodes ricinus*), as neuropeptides exert a crucial signaling function in the regulation of almost all physiological processes. In the experiment, 360 unfed female ticks were used, 240 were irradiated and 120 non-irradiated ticks represented the controls. Ticks were exposed to 900 MHz radiofrequency EMF with the intensities 2 V/m or 40 V/m at different duration of exposure time. Six neuropeptide genes and two receptor genes were examined by performing RT-qPCR on the cDNA from tick synganglia. A significant decrease in transcript levels of all of the tested genes in 40 V/m irradiation groups was found, especially after 1 hour and 3 hours of constant exposure. Upregulation of mRNA levels was noted in several genes after 2 V/m exposure, although only in *fgla/ast* significantly. For the genes *at*, *sifa*, *sif-r1*, *mip* and *mipr1* a significantly lower amount of transcripts after 1 hour of irradiation by 2 V/m was detected. A short-term high-intensity radiation therefore negatively affects transcription levels, while a more frequently occurring intensities (0.75 - 2 V/m) have a variable effect on tick neuropeptide expression. As EMR can affect the distribution and fitness of ticks, the research of physiology and behavior under irradiation is an important and novel research topic in acarology. Research funding: ANR-10-LABX-62-IBEID; ANR-21-CE14-0012; APVV-17-0372; APVV-19-0440; VVGS-2022-2192; VVGS-PF-2022-2135.



CUBA - 2024

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

9:30-10:00

Finding ticks and pathogens before they find us: DAMA protocol combined with citizen science

Gábor Földvári^{1,2}, Éva Szabó^{1,2}, Gábor Endre Tóth^{3,4}, Zsófia Tauber^{3,4}, Zsófia Lanszki^{3,4}, Brigitta Zana^{3,4}, Zsaklin Varga^{3,4}, Fanni Földes^{3,4}, Flóra Kulin^{1,2}, Domonkos Köves^{1,2}, Máté Miklós^{1,2}, Gábor Kemenesi^{3,4}

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The aim of our research is to implement the proactive DAMA (Document, Assess, Monitor, Act) protocol involving citizen-science methods. We started the TickWatcher project (<https://kullancsfigyelo.hu/en/>) in 2021 to monitor the appearance of non-indigenous ticks induced by climate change. Participants were asked to send us adult *Hyalomma* ticks, vectors of Crimean-Congo Haemorrhagic Fever Virus (CCHFV). Carried as immatures from Africa and Mediterranean areas via migratory birds, they have recently often emerged as adults in many European countries so far considered non-endemic. By intensive media outreach, our website quickly reached tens of thousands of people, and we received hundreds of ticks including a total of 14 *Hyalomma* specimens from over 300 participants. Sequencing confirmed morphological identification of the ticks. None of the specimens carried CCHFV, however high-throughput viral metagenomic sequencing identified the so far uncharacterized Volzhskoe tick virus, member of the *Bunyavirales* order. By providing the first genomic and phylogenetic characterization of this virus we enable future studies to gain deeper knowledge about the natural cycle and potential pathogenicity of this virus. Our work demonstrates that citizen science engagement combined with the DAMA protocol can effectively contribute to monitoring, and assessing the threats associated with introduced ticks and tick-borne pathogens and helps accomplish preventive measures.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens
Room La Corona, Meliá Cohiba

10:00-10:20

ZOONOTIC TICK-BORNE PATHOGENS IN SERBIA: WHAT WE KNOW AND WHAT WE DON'T KNOW?

Potkonjak Aleksandar¹, Savić Sara², Petrović Tamaš², Banović Pavle³, Ristanović Elizabeta⁴, Protić Đokić Vesna⁴, Pavlović Nevenka⁵, Kovačević Filipović Milica⁶, Jurišić Aleksandar⁷, Pustahija Tatjana⁸, Sević Siniša⁹, Poluga Jasmina¹⁰, Turkulov Vesna⁹

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In the past two decades, several research groups in major scientific, educational, health, veterinary and military institutions have intensively investigated the presence and distribution of zoonotic tick-borne pathogens in Serbia. These studies were carried out using molecular biology methods (conventional, real-time or microfluidic real-time PCR and partial sequencing) for tick pathogen investigation. It is important to point out that studies, apart from Tick-borne encephalitis virus (TBEV), were only and exclusively related to bacteria and protozoa. Thus, the presence of the following pathogens was identified in different population of ticks and in different geographical locations of Serbia: *Borrelia burgdorferi* s.s., *B. afzelii*, *B. garinii*, *B. lusitaniae*, *B. valaisiana*, *B. miyamotoi*, *Rickettsia monacensis*, *R. raoultii*, *R. massiliae*, *R. helvetica*, *Anaplasma phagocytophilum*, *Neoehrlichia mikurensis*, *Babesia microti* and *B. venatorum*. In addition to the applied methods of molecular biology, the successful cultivation of *Borrelia* spp. was also established (eg. *Borrelia afzelii* in BSK-H medium with antibiotics).

In addition to the research done on ticks, serological analysis were carried out in sentinel population of animals (eg. seroreactivity in dogs has been proven for *Borrelia burgdorferi* sensu lato complex, *Anaplasma phagocytophilum* and *Rickettsia conorii*). Some diseases from this group have also been registered in humans by infectious disease specialists in collaboration with microbiologists and epidemiologists (different clinical stages of Lyme borreliosis, which is expected because Serbia is an endemic area; and also rare cases of TBEV infection or TBEV seropositivity).

The causative agent of tick-borne encephalitis is relatively poorly investigated and there is a limited number of studies in Serbia. It was believed that TBEV was absent from the country, after the first registration in 1972. However, in recent years, the presence of TBEV has been confirmed in ticks (by PCR and partial sequencing) as well as in animals and humans (by serology tests).

Based on the above, it is evident that research on other viruses in ticks has not been carried out in Serbia so far, although more viruses with public health significance circulate in ticks such as the following ones: Omsk hemorrhagic fever virus and Kyasanur forest disease virus from genus *Orthoflavivirus*; Crimean–Congo hemorrhagic fever virus from g. *Orthonairovirus*; Bandavirus bhanjanagarensis (previous Bhanja virus) from g. *Bandavirus*; Thogotovirus thogotoense (previous Thogoto virus) and Thogotovirus dhoriense (previous Dhori virus) from g. *Thogotovirus*; and Eyach coltivirus (previous Eyach virus) from g. *Coltivirus*. Also, novel tick-associated viruses with unknown zoonotic potential have to be taken into consideration.

Therefore, we point out that in Serbia, the epidemiological situation regarding tick-borne viruses is not investigated extensively and the future research on tick-borne pathogens must highlight of viruses in ticks. The most useful data could be obtained with a metagenomic approach.

Key words: Tick-borne pathogens, zoonotic, viruses, bacteria, protozoa, Serbia



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

10:20-10:40

Adapting retrotransposon blood meal analysis (Rt-BMA) to identify tick hosts and assess tick feeding preferences in the United Kingdom

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Tickborne diseases pose an escalating threat to public health in the United Kingdom (UK), with Lyme disease, caused by *Borrelia burgdorferi*, being of notable concern. Understanding the feeding preferences of ticks is vital for elucidating their involvement in disease transmission dynamics and formulating effective control strategies. However, there is a lack of reliable tools for establishing tick feeding preferences. Retrotransposon blood meal analysis (Rt-BMA) has demonstrated efficacy in identifying tick hosts in the United States, yet its applicability in the UK remains unexplored. Retrotransposons, genetic elements capable of replication within genomes through retrotransposition, provide distinct sequences that can be leveraged to discern host species from tick blood meals. In this study, we adapted retrotransposon-based methodologies for use in the UK to identify various vertebrate species (including grey squirrels, voles, shrews, deer, and sheep) from tick blood meals. We evaluated Rt-BMA primers and probes using positive controls derived from blood samples of different species. Subsequently, we conducted fieldwork, collecting questing ticks from 14 woodland sites in Cumbria, UK, and extracting DNA from the blood meals within the ticks for Rt-BMA analysis. In addition to identifying various vertebrate species from tick blood meals, we also tested for the presence of Lyme disease by analysing ticks for *Borrelia burgdorferi*. Our study not only aims to establish comprehensive baseline data on the importance of different hosts for ticks, but also provides valuable insights into the prevalence of Lyme disease in tick populations. These findings are crucial for informing targeted interventions aimed at mitigating the risk of tick-borne diseases in the region.



CUBA - 2024

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

10:40-11:00

Tick-borne infections in Cuba: weaknesses and strengths for the application of One Health strategy from Human Public Health

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In Cuba the information about tick-borne infections in humans remains scarce; this is not the case in animals. Several exploratory and diagnostic studies have evidenced infection by *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, *E. canis*, *Babesia microti*, *Coxiella burnetii* and *Rickettsia* from the spotted fever group in exposed people. Genetic material of *Anaplasma* spp., *Babesia* spp., *Rickettsia amblyommii* and *Coxiella burnetii* has been also demonstrated in ticks, with emphasis on *Amblyomma mixtum*. For the prevention and control of this tick-borne pathogens is crucial the application of the One Health strategy. The application of different knowledge assessment tools in physicians and personnel exposed to tick bites, and clinical, epidemiological and laboratory studies related to tick exposed populations have made it possible to identify weaknesses and strengths to confront these pathogens with a vision of One Health. The insufficient knowledge about tick-borne diseases in physicians, the limitations in the acquisition of resources for the laboratory diagnosis, the low level of knowledge and risk perception of these illnesses and use of inadequate methods for the control and management of ticks in animal owners and caretakers threaten prevention and control, while alliances between leading institutions in the management of ticks and their pathogens, both in the human and veterinary areas, will contribute to confronting this health problem. Developing education and communication actions for people at risk, reinforcing relationships and communication between the human and veterinary sectors, specifically in tick-infested areas, will contribute to a greater approach to the epidemiological situation, which will result in better prevention and control of ticks and their pathogens.



CUBA - 2024

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

11:30-11:50

MALDI-TOF MS identification of ticks from North of Italy

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Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) has recently emerged as an alternative to morphological and molecular tools to identify tick species. In this study, we set out to evaluate and confirm the ability of MALDI-TOF MS to identify different species of ticks collected in the Northern region of Italy.

Ticks were collected and preserved in 70% ethanol or frozen. A total of 226 ticks were subjected to MALDI-TOF MS analysis to evaluate the intraspecies reproducibility and interspecies specificity of MS profiles obtained from the different species.

Morphologically, the ticks belonged to thirteen different species (*Argas reflexus*, *Rhipicephalus (Boophilus) annulatus*, *R. bursa*, *R. pusillus*, *R. sanguineus*, *R. turanicus*, *Dermacentor marginatus*, *Ixodes ricinus*, *I. hexagonus*, *I. acuminatus*, *I. canisuga*, *Haemaphysalis parva*, *H. punctata*).

Once the extraction method was set up, the IZSLER internal reference ticks library was created.

The spectra to be included in our database must satisfy two requirements:

- score ≥ 2 , which attests to excellent quality of the spectrum

- molecular confirmation by sequencing with 12S and/or 16S.

Currently, the MALDI-TOF library for the identification of ticks has a total of 68 spectra of 7 different species.

The library continues to be implemented with several new species, always following the same protocol: generation of a good quality spectrum and confirmation by sequencing.

In some cases, MALDI-TOF helped to solve some morphological misidentification of similar tick species.

In conclusion, MALDI-TOF MS represents an interesting approach to arthropod identification and has numerous advantages, including simplicity of specimen processing and very low consumable costs.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens
Room La Corona, Meliá Cohiba

11:50-12:10

Phylogenetic inferences based on distinct molecular markers reveals a novel *Babesia* (*Babesia pantanalensis* nov. sp.) and a *Hepatozoon americanum*-related genotype in crab-eating foxes (*Cerdocyon thous*)

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Abstract

Piroplasmids and *Hepatozoon* spp. are tick-borne apicomplexan protozoa that can cause disease in several canid species. The present study aimed to expand the knowledge on the diversity of piroplasmids and *Hepatozoon* in crab-eating foxes (*Cerdocyon thous*; n=12) sampled in the Pantanal of Mato Grosso do Sul State, central-western Brazil. PCR assays based on the 18S rRNA were used as molecular screening for piroplasmids and *Hepatozoon* spp. Three (25%) and 11 (91.7%) were positive for piroplasmids and *Hepatozoon* spp., respectively. Co-infection was found in three *C. thous*. Phylogenetic analyses based on the near-complete 18S rRNA, *cox-1* and *hsp70* genes evidenced the occurrence of a novel of *Babesia* spp. (namely *Babesia pantanalensis* nov. sp.) closely related to *Rangelia vitalii* and *Babesia* sp. 'Coco'. This finding was supported by the genetic divergence analysis which showed (i) high divergence, ranging from 4.17 to 5.62%, 6.19 to 6.20% and 4.91 to 9.25% between the 18S rRNA, *hsp70* and *cox-1* sequences detected in this study and those closely related and (ii) the genotype network (which displayed genotypes detected herein separated from other *Babesia* species and *Rangelia* by median vectors and several mutational events). Also, phylogenetic analysis based on the 18S rRNA gene of *Hepatozoon* spp. positioned the sequences obtained herein in a clade phylogenetically related to *Hepatozoon* sp. 'Curupira 2', *Hepatozoon* sp. detected in domestic and wild canids from Uruguay and *Hepatozoon americanum*. The present study described *Babesia pantanalensis* nov. sp. and *Hepatozoon* closely related to *H. americanum* in crab-eating foxes from Brazil. Moreover, the coinfection by piroplasmids and *Hepatozoon* sp. showed for the first time in crab-eating foxes strongly suggest that this wild canid species potentially acts as a bio-accumulator of hemoproteoza in the wild environment.

Keywords: molecular diagnosis, apicomplexan protozoa, *Babesia*, *Hepatozoon*, crab-eating foxes, phylogeny



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

12:10-12:30

Hard ticks from Iberian wolves (*Canis lupus signatus*) and their associated microorganisms, North of Spain

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Introduction/objective: Studies on the ecology and health of the grey wolf (*Canis lupus*) are numerous, but little is known about the ticks they host. This study aimed to identify the ticks and their microorganisms from Iberian wolves (*Canis lupus signatus*) in northern Spain (Europe).

Methods: From 2017 to 2023, ticks removed from wolf carcasses at the CWC were classified and processed at CRETAV. Nucleic acid extracts obtained from pooled ticks were analyzed using specific-PCR assays for detecting: *Borrelia*, *Rickettsia*, *Anaplasma*/*Ehrlichia*/*Neoehrlichia*, *Francisella*, *Babesia*/*Theileria*/*Hepatozoon*, flaviviruses, orthonairoviruses and phenuiviruses.

Results: A total of 392 ticks removed from 72 wolves were grouped in 93 pools. Ticks belonged to five species (Tick-No./Pool-No.): *Dermacentor reticulatus* (85/32), *Haemaphysalis coccina* (24/6), *Ixodes hexagonus* (14/3), *Ixodes ricinus* (262/48) and *Rhipicephalus bursa* (7/4).

D. reticulatus pools tested positive results for *Rickettsia raoultii* (n=29), *Francisella* spp. (n=31), *Babesia caballi* (n=1) and *Uukuvirus* spp. (n=23). *Candidatus* *Rickettsia kotlanii* was amplified from one *H. coccina* pool. *Wolbachia* sp. and *Ixovirus* spp. were detected in one and two *I. hexagonus* pools, respectively. Microorganisms amplified from *I. ricinus* pools were: *Borrelia lusitaniae* (n=1), *Borrelia miyamotoi* (n=1), *R. raoultii* (n=1), *Candidatus* *Mitochondria mitochondrii* (n=11), *Anaplasma phagocytophilum* (n=7), *Alloccryptoplasma* sp. (n=1), *Hepatozoon canis* (n=2) and *Ixovirus* spp. (n=4). One *Ehrlichia canis*, one *Hepatozoon martis*, three *Uukuvirus* spp. and three *Mivirus boeae* amplicons were found in *R. bursa* pools. Other microorganisms were not detected.

Conclusions: *D. reticulatus*, *H. coccina*, *I. hexagonus*, *I. ricinus* and *R. bursa* are common ectoparasites of the wolves in northern Spain.

The Iberian wolf is involved in the epidemiology of tick-borne pathogens of veterinary and zoonotic importance.

The circulation of *Ca. R. kotlanii* and *Alloccryptoplasma* spp. is first reported in western Europe. This is the first study of viruses in ticks from wolves and it demonstrates the presence of novel tick-borne viruses in Spain.

Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens

Room La Corona, Meliá Cohiba

12:30-12:50

***Dermacentor* ticks and human rickettsioses: a parallel expansion in Eastern France ?**

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Ticks and human tick-borne diseases are worldwide in expansion, especially in the temperate zones of the northern hemisphere. While this expansion is well-documented for the complex *Ixodes ricinus*, *Dermacentor* spp. which is the second most abundant tick in Europe is less investigated.

We selected Eastern France, highly endemic for ticks to identify the ecosystem the most conducive for *Dermacentor*. Since this tick can transmit *Rickettsia*, we then analyzed their infection rate for *R. slovaca* and *R. raoulti*, the causative agents of a human rickettiosis called TIBOLA (Tick-BORne LymphAdenopathy), and in parallel we collaborated with clinicians to evaluate the number of clinical cases in this region.

Two species of *Dermacentor* are present: *D. reticulatus* and *D. marginatus*. The most represented is *D. reticulatus* (94%). It is not surprising since *D. marginatus* is a more xerophilic tick. We then run a PCR to detect *Rickettsia*: 85 % of ticks were infected by *R. raoulti* and 15% *Rickettsia* spp.. Concerning the ecosystem, these ticks were mainly collected in wetlands and alluvial forests, in places where wild boars are present or domestic animals (dogs and/or horses). The main peak of tick activity was March-April, a smaller peak in September.

In parallel, the clinicians did a retrospective study to measure whether *Dermacentor* was more at risk of *Rickettsia*-transmission these last years (2016-2021). Most cases of TIBOLA (15) appeared in 2021 with 1 to 3 cases a year from 2016 to 2020. However, it must be considered that the number of cases are likely underestimated since East of France is highly endemic for Lyme borreliosis and many clinicians are not familiar with *Dermacentor* tick and their infection with *Rickettsia*.

Due to the modifications of the ecosystem with higher biodiversity and changes of human practices, the awareness of people and clinicians is essential for a better prevention.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

12:50-13:10

Ticks around and in Paris: presence, pathogens and risk-maps

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Ticks are hematophagous acarids known to be important vectors of several pathogens of medical and veterinary importance, such as *Borrelia* spp., *Babesia* spp., or tick-borne encephalitis virus. The geographical distribution of these vectors is impacted by environmental, socio-economical and climate changes, which can lead to the emergence of tick-borne diseases. In this context, the trend for urban greening can provide suitable environments for ticks and their hosts, and with promotion of outdoor activities for a healthier lifestyle, the risk of encountering ticks and their pathogens for humans will increase. The LabEx project "Microbiological monitoring: new horizons for tick-borne diseases" has been built upon this background. It aims to provide evidence-based recommendations and tick risk maps for the general public on the green spaces in and around Paris (France). For that purpose, questing ticks were collected in both spring and autumn 2022 and 2023 by flagging vegetation. Tick collections were performed in 166 sites of 32 different green areas of Ile de France: urban parks, peri-urban woods, green infrastructures and forest.

In total, 3456 ticks were collected, most of them being *Ixodes ricinus*. Ticks were mainly detected in forest but also in periurban woods. Despite being a less suitable environment for tick population establishment, urban parks and green infrastructures are still at risk, as ticks were also collected in these areas. The environment is a key parameter in the distribution of ticks and hence tick bite risk and pathogen transmission. Detection of various pathogens (parasites, bacteria and viruses) in collected ticks is ongoing and prevalence rates will be added to the maps to enable assessment of the risk of tick-borne diseases. Space frequentation and human mobility will also be added to provide a population level estimates of the burden of risk.

Keyword: tick-borne pathogens, urbanisation, green spaces, risk maps.

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

13:10-13:30

Ticks' diversity and distribution in Saudi Arabia, with insights from ecological niche modeling approaches

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Ticks, as hematophagous ectoparasites, play a significant role in the transmission of various pathogens to animals and human. This study focuses on understanding the diversity and distribution of ticks in Saudi Arabia, a region with substantial importance in global energy reserves and increasing socio-economic changes. Ticks pose a considerable threat to livestock, human health, and the overall economic well-being of the population. With the prevalence of tick-borne diseases on the rise, this research explores the implications for public health, livestock management, and the broader ecological landscape. A comprehensive dataset was assembled through an extensive literature search conducted on Google Scholar and PubMed databases, covering reports on tick distributions in different regions of Saudi Arabia from 1960 to 2023. The data extraction process included tick species names, collection coordinates, locations, and time of collection. Geospatial data were extracted from Google Earth and Google Maps, and ecological niche modeling was performed using various algorithms to understand current and future tick distributions. Environmental variables were incorporated, and the accuracy of models was assessed using the area under the curve index.

The dataset comprises 258 geospatial records representing 35 unique tick species from six genera. The study reveals an increasing trend in tick reports over the years, with the predominant genera being *Hyalomma* (*Hy*) and *Rhipicephalus* (*Rh*). *Hy. dromedarii*, *Hy. impeltatum*, and *Rh. turanicus* emerge as major contributors to tick diversity. Ecological niche modeling demonstrates good to excellent accuracy for various tick genera, with implications for disease transmission dynamics. However, the findings of this study provide crucial insights into the diversity, distribution, and ecological niches of ticks in Saudi Arabia. The identified trends have implications for public health, livestock management, and environmental conservation.



CUBA - 2024

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

15:00-15:20

The Phenology of Ticks and Tick-borne Pathogens in a University Green Zone in Georgia, USA

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Ixodid ticks are commonly found in various wildlife habitats across Georgia, USA. However, little is known about the phenology of ticks within green zones in small urbanized areas. The purpose of this study was to determine the seasonal distribution and abundance of ticks and their carriage of rickettsiae on a nature preserve and adjacent walkways of the university campus. Ticks were collected weekly by flagging or dragging 17 sites from June 2022 through March of 2024, and ambient parameters were recorded. Ticks were identified to species, sex and life stage using standard keys. DNA was extracted and 12S tick mitochondrial DNA fragment was amplified. SYBR-Green PCR was used to test ticks for spotted fever group *Rickettsia* (SFGR) and *Anaplasmataceae* followed by amplification and sequencing of *ompA* and *groEL* fragments to identify the respective organisms detected. Strain typing was done by analyzing sites with variable numbers of tandem repeats. 697 ticks were collected including 216 *Amblyomma americanum* (18.1% females, 19.9% males, and 62.5% nymphs), 433 *Amblyomma* sp. larvae, 38 *Ixodes scapularis* and 9 *I. affinis*. Tick abundances correlated with ambient temperature but did not depend on rainfall, humidity or air quality. *Amblyomma americanum* was collected from March through October, *I. affinis* was found from August through October, and *I. scapularis* was found from November through March. SFGR DNA was detected in 36.5% of ticks (66 *A. americanum* and 7 *Ixodes* sp. ticks) and 69.9% of larval pools. Only *Rickettsia amblyommatis* was identified in *Amblyomma* ticks, *Ixodes* sp. ticks contained *R. buchneri*. Eleven *Amblyomma* ticks testing PCR-positive for *Anaplasmataceae* contained *Ehrlichia chaffeensis* (3.8%), *E. ewingii* (1.3%) and Panola Mountain *Ehrlichia* sp. (1.3%). In conclusion, ticks and tick-borne rickettsiae can be readily found within small-scale green areas of a university and may pose risks of tick-borne infections for individuals using these places.

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

15:20-15:40

Ecology and life cycle of *Otobius megnini* (Dugès, 1884) in Central Mexico

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The soft tick, *Otobius megnini* (Dugès, 1884), is known as the spinose ear tick because larvae and nymphs are found withing ears of several mammals including cattle, horses, sheep, goats, dogs and cats. This tick was reported for the first time in Mexico in 1884. However, studies on its ecology and biology have not been performed. The aim of this study was to determine the ecology and life cycle of *O. megnini* in cattle from Central Mexico. Ticks were collected in two farms from El Marques in the state of Queretaro, situated in the Mexican plateau at 1850 meters above the sea level, latitude 20.31°N and longitude 100.09°W. Three Charolais cattle from each farm were checked for ticks. All animals were found positive and larvae and nymphs in several stages of engorgement were collected. In average, 85 ticks per animal were found. The highest infestations (124 ticks per animal) were found in young animals. Ticks were cleaned, identified (Only *O. megnini* were found), and incubated for molting. Molting from nymphs into adults occurred in 5 to 10 days. Mating occurred soon after molting. Oviposition occurred after 12 to 14 of mating. Unlike hard ticks, *O. megnini* intermittently laid eggs without forming egg masses. Eggs hatched after 12 to 14 days of incubation, and larvae remained alive and active during 45 days. Studies are ongoing to complete the life cycle, to establish the biological parameters, and to determine the population dynamics of *O. megnini*. These studies will allow the establishment of control programs of this soft tick in the region.

Key words: Soft tick, *Otobius megnini*, life cycle



CUBA - 2024

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

15:40-16:00

Clinical, epidemiological and serological findings in Cuban individuals bitten by ticks

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Abstract:

Rickettsiosis and Q fever have a wide geographical and temporal distribution associated with the presence of ticks and local fauna. Several species of *Rickettsia* have been described that cause fever combined with an exanthematous rash. *Coxiella burnetii* is described as the causative agent of Q fever, characterized by high fever, headache and muscle pain, among other symptoms. These zoonoses have not been reported in Cuba, but they are of special interest in areas where the rate of tick infestation is high. The objective of this work was to obtain clinical, epidemiological and serological evidence of both diseases in a rural Cuban population affected by tick bites. A clinical evaluation, epidemiological surveys, entomological studies and indirect immunofluorescence tests for the detection of IgM and IgG to spotted fever group rickettsia and *C. burnetii* were carried out. The clinical evaluation showed multiple tick bites, mainly on the lower limbs, upper limbs and abdomen, reporting persistent itching. The patients did not present any other symptoms that suggested *Rickettsia* or *Coxiella* infection. The epidemiological survey showed different occupational categories, the most relevant being agricultural and livestock work. Several species of ticks of medical importance were identified such as: *Amblyomma mixtum*, *Rhipicephalus microplus* and *Dermacentor nitens* as well as the presence of IgM and IgG antibodies to rickettsia of the spotted fever group and *C. burnetii* in the individuals studied. The findings show the exposure of persons to etiological agents although the development of the specific disease has not been demonstrated, which suggests the need to maintain epidemiological surveillance with a One Health approach.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

16:00-16:20

**Tick-borne pathogen distribution in tick tissues collected from dromedary camels in Kenya:
Predicting vector competence**

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Surveillance studies on tick-borne pathogens (TBPs) frequently use whole-tick homogenates to understand associations between ticks and pathogens. However, understanding the distribution of TBP infection within specific tick tissues (saliva, hemolymph, salivary glands, and midgut) can help unravel pathogen transmission mechanisms and disentangle pathogen detection from vector competence. We screened for *Anaplasma*, *Ehrlichia*, *Coxiella*, *Rickettsia*, *Theileria*, and *Babesia* pathogens by PCR-HRM analysis of 278 camel blood samples and 504 tick tissues derived from 126 camel ticks sampled in Kenya (Laikipia and Marsabit counties). *Candidatus Anaplasma camelii* infections were prevalent in camels (91%) yet absent in all ticks (*Rhipicephalus pullchelus*, *Amblyomma gemma*, *Hyalomma dromedarii* and *Hyalomma rufipes*). We detected *Ehrlichia chaffeensis*, responsible for human monocytic ehrlichiosis, for the first time in the blood of one camel. *Ehrlichia ruminantium* was detected in all tissues of the four tick species. *Rickettsia africae* exhibited the highest prevalence in *Am. gemma* (62.5%), primarily in the hemolymph (45%) and less frequently in the midgut (27.5%). Conversely, the lowest occurrence of *R. africae* was observed in *Rh. pulchellus* (29.4%) midgut (17.6%) and hemolymph (11.8%). Similarly, *R. africae* was predominantly detected in the midgut of *Hyalomma dromedarii* (41.7%) but was absent in the hemolymph. *Rickettsia aeschlimannii* was found only in *Hy. rufipes*, mainly in the hemolymph (80%), which is congruent with the role of this tick species as its transmission vector. No *Coxiella*, *Theileria*, or *Babesia* spp. were detected. Our findings suggest that presence of TBPs in tick hemolymph may serve as an indicator of vector competence, particularly in comparison to detection in the midgut from which they must cross tissue barriers for effective replication and dissemination across tick tissues. More studies should focus on exploring the distribution of TBPs within tick tissues to enhance knowledge of TBP epidemiology and to distinguish competent vectors from dead-end hosts.

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

16:20-16:40

Which pathogens can the neglected winter ticks (*Haemaphysalis inermis*) carry?

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Our study aimed to broaden our understanding of the diversity and prevalence of pathogens carried by the winter tick (*Haemaphysalis inermis*). Previous research has shown that *H. inermis* might act as a vector for tick-borne encephalitis virus and may also transmit other pathogens to humans (e.g., *Rickettsia aeschlimannii*, *Rickettsia helvetica*) and livestock (*Babesia bigemina*). However, the full extent of *Haemaphysalis inermis* role as a vector and the range of pathogens it can spread remain poorly understood. *Haemaphysalis inermis* ticks are widely distributed across Eurasia and can bite humans, yet the species is relatively understudied. It is particularly common in Central European forests from November to February. Misconceptions about decreased tick activity during colder months may lead individuals enjoying winter outdoor activities to underestimate risks and warmer winters might attract more people to spend their free time in the forests. This highlights the need for accurate data on the vector potential of this species. During our study we processed 208 *Haemaphysalis inermis* ticks (123 females, 85 males) using Reverse Line Blot (RLB) to detect carried pathogens over two years (October 2022 to January 2023 and October 2023 to December 2023). Preliminary findings are intriguing: 48% of ticks showed a positive signal for *Rickettsia* using the catch-all (16s rRNA) probe, primarily *Rickettsia helvetica*. Some samples exhibited catch-all signals without detection of specific common *Rickettsia* species, suggesting potential rare or unidentified species. *Babesia* species were also common (33% detection rate with *Babesia microti* predominating), along with *Borrelia* and *Anaplasma* species in around 10% of samples. The diversity and abundance of these pathogens have surpassed our expectations. Moving forward, our research aims to further characterize *H. inermis* ticks and their role as vectors. By identifying the pathogens they carry, we can better understand associated health risks and inform decision makers amid changing environmental conditions.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

16:40-17:00

A dataset of ticks and tick-borne pathogens of Pakistan raises biosecurity concerns for the country

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Abstract

Tick the 2nd most important vector after mosquito is a hematophagous ectoparasite of humans, animals, reptiles, and birds. It is responsible for the transmission of various infectious disease-causing agents including bacteria, protozoan parasite and viruses. The current study is aimed to analyze ticks and its associated pathogens and viruses reported from Pakistan till 2023. Relevant literature was retrieved from PubMed, Scopus, Web of Science, and Science Direct using keywords such as "Pakistan," "ticks," "tick-borne pathogens," and "tick-borne viruses." Duplicate citations were removed using EndNote 20. Articles reporting ticks and their associated pathogens isolated directly from ticks were selected for this study. The findings reveal Pakistan's unique tick fauna, hosting 46 different tick species belonging to 9 genera (namely *Amblyomma*, *Argas*, *Dermacenter*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Nosomma*, *Ornithodoros*, and *Rhipicephalus*), mainly reported after 2010. The genera *Hyalomma* and *Rhipicephalus* were found to be abundant in the study areas. Recent reports of *Ixodes* species, previously non-endemic to the area, raise biosecurity concerns for tick-borne diseases. These ticks prevalent in any climatically distinct areas from colder climatic regions to more warmer regions with high number of tick inhabiting warmer regions. The pathogen profile of these tick species provides insights into the region's reported pathogen diversity, primarily bacterial and parasitic microorganisms, with few reports of viruses. In total, 37 unique pathogen species have been reported from ticks infesting humans, animals, reptiles, and birds in Pakistan. The study underscores the increasing reports of ticks over the past decade, raising concerns for wildlife and human health. This study offers fundamental information on the tick fauna and its associated pathogens in Pakistan emphasizing the need for enhanced surveillance and preventive strategies to mitigate the public health impact of tick-borne diseases.

Keywords: Pakistan; Ticks; Tick-borne Pathogens; Biosecurity; Health Implications; Tick-borne Diseases



CUBA - 2024

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

9:30-10:00

High-throughput nanotechnologies for tick-borne pathogens detection

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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 (TBPs), 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 TBPs in different countries. Those new tools also demonstrated their ability to study tick co-infection and genetic diversity of TBPs. Moreover, to analyze the virome of ticks (*Ixodes ricinus*, *Amblyomma variegatum* and *Rhipicephalus microplus*), a complementary approach using high-throughput sequencing (HTS) were performed to allow the detection of new and unexpected viruses present in ticks.

These complementary new high-throughput surveillance methods allow us to detect and characterize a high number of TBPs, particularly new ones. They also represent a major improvement in epidemiological studies, able to facilitate comprehensive testing of TBPs in ticks, mammals and humans, and which can also be customized to monitor emerging diseases.

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

10:00-10:20

***Midichloria mitochondrii* stimulates the sylvatic cycle of Lyme spirochetes in *Ixodes ricinus* instars**

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Abstract

Ixodes ricinus nymphs carrying the vertically transmitted *Midichloria mitochondrii* (*Midichloria*) are more often infected with the horizontally transmitted *Borrelia burgdorferi* sensu lato (*Borrelia*), and female nymphs take a larger bloodmeal than males. Also, female adult ticks have more often *Midichloria* than males. Here, we aimed to disentangle the role of the sex and *Midichloria* presence in tick instars in the sylvatic cycle of *Borrelia*. For this, we developed a qPCR for the specific detection of male *I. ricinus*.

There was no difference in the infection rate of *Midichloria* between male and female larvae, and neither its presence nor the sex affected the size of larvae. However, the infection with *Midichloria*, not the sex, increased the uptake of *Borrelia* in larvae feeding on *Borrelia*-infected birds. This aligns with the finding that the infection with *Borrelia* in questing nymphs was positively associated with *Midichloria*, but not with their sex. Remarkably, female nymphs were more (often) infected with *Midichloria* than males. Both the presence of *Midichloria* and the (female) sex were positively associated with the size and weight of nymphs. We further corroborate that the infection rates of *Midichloria* in ticks shows substantial spatial variation, and now show that locations with a higher *Midichloria* prevalence in nymphs have a significantly higher infection rate with *Borrelia*.

Thus, *Midichloria* alters the propensity of *I. ricinus* instars to facilitate the sylvatic cycle of *Borrelia*, and is therefore an additional factor that contributes to the spatial variation in Lyme disease risk.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

10:20-10:40

Tick-borne parasites in a high-throughput analysis era: usefulness of MALDI-TOF and Real-Time Microfluidic PCR in the study of parasite distribution in domestic animals and urban wildlife

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Abstract.

The increasing abundance and distribution of hard ticks within Europe have been reported extensively over the last 10–20 years. Tick populations in urban and peri-urban environments are increasingly recognized as significant vectors for a wide array of pathogens that not only threaten the health of domestic animals but also pose a risk to public health. Urban wild animals are suitable hosts for ixodid vectors, and therefore they may play a role as reservoir of vector-borne pathogens.

MALDI-TOF MS has recently been proposed as an accurate tool for arthropod identification, and this includes hard ticks' identification. Urban wildlife is often parasitized with a wide species range of hard ticks. We decided to construct a main spectra library of ixodid ticks. For this purpose, we used specimens recovered from urban wild mammals (hedgehogs, roe deers and foxes) hosted at the Wildlife Hospital of the Veterinary College of Alfort (EnvA), in Paris, France. We succeeded to create an MSP database for the identification of *Ixodes hexagonus* and *I. ricinus*. The establishment of an *Ixodes* MSP bank at EnvA will allow further studies in wildlife or domestic hosts using a method considered faster and cheaper than conventional methods. This approach will increase the possibilities of vector identification by transporting specimens simply in ethanol, and will represent a valuable tool for ecological and epidemiological studies.

Furthermore, we have started a preliminary study that aims to contribute to a better knowledge regarding the circulation of tick-borne pathogens in ticks recovered from urban wild mammals from Paris region. The presence of these pathogens in the blood meal ticks engorged on wild animals will provide insights into the role of urban wildlife as reservoir/sentinels for infectious diseases transmissible to domestic animals or to humans. Also, we assessed the circulation of tick-borne pathogens in urban wild mammals by blood sampling and high-throughput molecular screening.

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room Internacional I, Meliá Internacional Varadero

10:40-11:00

Occurrence and genetic identity of *Babesia* spp. in deer and cats in Poland

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Babesiosis is an emerging infectious disease in Europe which affects domestic animals, livestock, wildlife and humans. Different deer species are known reservoir of several *Babesia* species, including zoonotic *Babesia divergens*. Studies on *Babesia* and other piroplasm in cats in Europe are rare and incomplete.

The aim of the current study was to determine molecular identity of babesiae infecting free-living deer species and free-roaming cats in Poland. Piroplasm species was determined by PCR amplification and sequencing of 18S rRNA gene fragment, followed by phylogenetic analysis.

Three *Babesia* spp. were identified among deer, including *B. divergens* and newly identified *B. odocoilei*-like ‘deer genotype’ of *Babesia*. Surprisingly, only *Babesia canis* vectored by *Dermacentor reticulatus* ticks was identified among cats, including asymptomatic individuals and individuals treated because of babesiosis.

Conclusion: deer in Poland can be infected with several species of deer-specific babesiae, however infections in cats are mostly due to canine piroplasm, *B. canis*.

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CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

11:30-11:50

First detection of Crimean–Congo haemorrhagic fever virus in *Hyalomma marginatum* ticks, southern France.

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Ticks, which are vectors of several zoonotic pathogens, represent an important and increasing threat for human and veterinary health. *Hyalomma marginatum*, one of the main tick vectors of the Crimean-Congo Haemorrhagic Fever (CCHF) virus, has been present in Corsica for decades. Given the recent establishment of this tick species in continental France, it was crucial to evaluate the epidemiological situation of CCHFV in France. Transmission of the CCHFV to humans occurs predominantly via bites of *Hyalomma* ticks, especially *H. marginatum* and *H. lusitanicum* in Europe, or via exposure to infected blood or tissues from viraemic animals or humans. As ticks are the only known natural reservoirs of CCHFV, we focused on field collection of ticks. We collected ticks from horses, which are considered to be the preferred hosts of *H. marginatum* and on cattle, which are considered as good amplifiers of CCHFV and thus enhance local virus circulation. We collected ticks in the Pyrénées-Orientales department where antibodies against CCHFV were identified in 2021-2022 from cattle. Cattle farms with the highest within-herd seroprevalences were selected, as well as a few seronegative farms in the same areas. In addition, farms with horses, located in the neighbourhood of the seropositive cattle farms were also visited. The ticks *H. marginatum*, analyzed molecularly, revealed the presence of CCHFV in this department, in proportions ranging from 3.1% to 55.8% of infected ticks across positive sites. All CCHFV isolates sequenced in this study were highly identical and belonged to the same genotype (genotype III). This finding confirms for the first time the transmission of CCHFV in France and highlights the need for close monitoring of *H. marginatum* in areas where the tick is already established, and further investigations into its probability of geographic expansion.



CUBA - 2024

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

11:50-12:10

Detection of *Ixovirus* spp. in ticks collected from mink in Spain.

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Introduction/objective: The main threat to critically endangered European mink (*Mustela lutreola*-EM) is the American mink (*Neogale vison*-AM) competition. Mink are important hosts of ticks, but their role in the epidemiology of tick-borne diseases remains unknown. Our preliminary data of the largest study of ticks from mink showed bacteria (*Ehrlichia* spp., *Candidatus* *Neoehrlichia mikurensis*, *Coxiella* spp., and *Rickettsiella* spp.) and protozoan (*Hepatozoon martis*) infections without evidence of tick-borne viruses' amplification (presented at ESCCAR Congress, Lausanne 2022). This test aimed to improve the tick-borne viruses detection in these mink ectoparasites.

Methods: Fifty cDNA samples corresponding to 61 *Ixodes acuminatus* (11 pools) and 286 *Ixodes hexagonus* (39 pools) collected from EM and AM in Spain, were reanalysed through a semi-nested Pan-Phenuivirus PCR assay (L segment, *RdRp*). This tool was designed with the same primer pairs of single PCRs previously used.

Results: Phenuiviruses were amplified from nine female tick pools, three *I. acuminatus* (IA, two genotypes) and six *I. hexagonus* (IH, five genotypes). All the sequences showed the highest identities (nucleotide: 76.4-87.7%; amino acids: 81-98.1%) with those corresponding to the *Ixovirus* genus. Following the species demarcation criteria (*RdRP* amino acid sequence >95%), only one IH genotype was identified at the species level, as *Ixovirus norvergiae*. Phylogenetic analysis showed two clusters close to *Ixovirus ixodis*, one containing the IA genotypes and the other with three IH genotypes. The remaining IH genotype belonged to *Ixovirus* spp. but independent and far from all known species.

Conclusions: This is the first detection of Phenuiviruses in *I. acuminatus* and *I. hexagonus* worldwide, including new virus species.

This is the first report of *Ixovirus* spp. in Spain.

Zoonotic potential of these viruses and the role of mink in the epidemiology of tick-borne viruses should be investigated.

The scope of tick-borne viruses may be underestimated.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

12:10-12:30

ONE HEALTH IN INDIGENOUS TERRITORIES: DOGS AS SENTINELS FOR TICK-BORNE DISEASES

Liliane Silva Durães^{1*}, Lucas Belchior Souza de Oliveira²; Andressa de Oliveira Silva²; Tayanne Moreira de Vete Lima²; Gabriela Ferreira Félix²; Maria Luiza da Cunha Cabra²; Ana Iris De Lima Dure³; Marciel Xakriabá⁴; Marcelo Pires Nogueira de Carvalho⁵; Danielle Ferreira de Magalhães Soares²; Júlia Angélica Gonçalves da Silveira²; Camila de Valgas e Bastos Castro²; Camila Stefanie Fonseca de Oliveira²

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Xakriabá Indigenous Land (XIL) is the largest indigenous population in the state of Minas Gerais, Brazil. XIL has historically faced inadequate attention to public health, particularly in terms of basic sanitation and zoonotic diseases occurrence. Domestic dogs are commonly found and often infested with ectoparasites. The purpose of this study was to investigate the frequency of tick-borne diseases (TBD) in dogs in the XIL and its associated risk factors. During the rainy season, a total of 87 dogs from eight indigenous villages were examined and blood samples were collected. Nested PCR technique was employed for molecular testing, with primers designed to detect hematopathogens. Additionally, 42 samples were analyzed by serological testing through Indirect Immunofluorescence Assay. The dog's physical examination included body condition score, lymphadenopathy, mucosal coloration, clinical changes, presence of ectoparasites, and use of an ectoparasiticide. Those factors were associated with TBD. Molecular testing revealed that 29% (25/87) tested positive for monocytic *Ehrlichia*, 49% (42/85) for *Babesia/Hepatozoon* (BH) and 20% (17/87) for granulocytic/platelet Anaplasmatidae (EG). Among the 17 animals positive for EG, 35% (6/17) were also positive for specific reaction to *Anaplasma phagocytophilum*, while among the 42 positives for BH, 14% (6/42) were positive for specific reaction to *Hepatozoon canis*. It was observed that not using a traditional ectoparasiticide increased the odds of BH positivity by 3.2 times (95% CI [1.03, 10.1]). Serological testing showed 55% (23/42) of the samples tested positive for *A. phagocytophilum* and 72% (32/42) tested positive for *Ehrlichia canis*. Notably, dogs infected with *A. phagocytophilum* had almost five times higher odds of lymphadenopathy (95% CI [1.09, 22.36]). These findings highlight the presence of emerging diseases with implications for animal and human health within indigenous territory. They underscore the importance of active disease surveillance to prevent larger-scale health issues and emphasize the relevance of One Health approach.

Key-words: Hemopathogens, emerging diseases, molecular diagnosis, serological diagnosis, indigenous veterinary medicine, traditional multi-species families.

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CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

12:30-12:50

Molecular detection and characterization of tick-borne Anaplasmataceae agents in vampire bats in the Brazilian Amazon

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Abstract

Although bats (Mammalia: Chiroptera) act as natural reservoirs for many zoonotic pathogens around the world, few studies have investigated the occurrence of Anaplasmataceae agents in bats, especially vampire bats. The Anaplasmataceae family (order Rickettsiales) encompasses obligate intracellular bacteria of the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia*, *Neoehrlichia* and *Wolbachia*. The present study aimed to investigate, using molecular techniques, the presence of *Anaplasma* spp. and *Ehrlichia* spp. in vampire bats sampled in northern Brazil. Between 2017 and 2019, spleen samples were collected from vampire bats belonging to the species *Desmodus rotundus* (n = 228) and *Diaemus youngii* (n = 1) from the states of Pará (n=207), Amazonas (n=1), Roraima (n=18) and Amapá (n=3). Positivity rates of 5.2% (12/229) and 3% (7/229) were found in PCR assays for *Anaplasma* spp. (16S rRNA gene) and *Ehrlichia* spp. (*dsb* gene), respectively. The present study revealed, for the first time, the occurrence of *Anaplasma* sp. and different genotypes of *Ehrlichia* spp. in vampire bats from Brazil. While phylogenetic analyzes based on the *dsb* and *ftsZ* genes of *Ehrlichia* and 16S rRNA of *Anaplasma* spp. revealed phylogenetic proximity between genotypes detected in vampire bats and Anaplasmataceae agents associated with domestic ruminants, phylogenetic inferences based on the *gltA* and *groEL* genes evidenced the occurrence of genotypes apparently exclusive to bats. This is the first molecular detection of *Ehrlichia* and *Anaplasma* in vampire bats.

Keywords: *Anaplasma* spp., *Ehrlichia* spp., Chiroptera, *Desmodus rotundus*.

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

12:50-13:10

Prevalence and predictors of tick-borne pathogens in deer communities

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Background: It is expected that varying vertebrate communities affects the presence, prevalence and diversity of ticks and their pathogens. How underlying mechanisms, such as amplification, dilution and spill-over, interact and generate the pathogen communities is understudied. We aimed to investigate this by determining the prevalence of vector-borne parasites, bacteria and viruses in four different deer species with varying their community composition across the United Kingdom.

Methods: From January 2020 to May 2021, blood samples of ~2400 deer from across six species were collected from across Great Britain. These are being analysed and typed by polymerase chain reaction (PCR) for the presence of genetic material from *Anaplasma phagocytophilum*, *Bartonella* spp., *Babesia* spp., *Borrelia burgdorferi* sensu lato (s.l.), *Borrelia miyamotoi*, *Coxiella burnetii*, *Francisella tularensis*, Grotenhout virus, *Hepatozoon* spp., *Midichloria mitochondrii*, *Neoehrlichia mikurensis*, *Rickettsia* spp., *Rickettsiella* spp., *Spiroplasma ixodetis*, *Trypanosome* spp., and Uukumeni-virus. Our approach allows for the detection of multiple *Anaplasma* ecotypes and *Babesia* species in singular samples. Serology was also done for tick-borne encephalitis virus. The possible associations of host factors, such as species, relative densities, age category, sex, nutritional condition and health status, as well as several environmental variables will be used as potential explanatory variables.

Results and conclusion: So far, ~350 samples have been analysed and we have detected DNA from *A. phagocytophilum* ecotype I and II, *B. schoenbuchensis*, *Ba. divergens*, *Ba. venatorum*, *Ba. capreoli*, *Ba. odocoilei*, *F. tularensis*, *M. mitochondrii*, *Rickettsia* spp., *Rickettsiella* spp., and *T. melophagium*. Deer were frequently infected with two *Babesia* species, and occasionally with two *Anaplasma* ecotypes. Surprisingly, the RNA of two segments of the Grotenhout virus was found in 4 individuals. Analyses are still ongoing and will be presented at the conference.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

13:10-13:30

Molecular detection of *Rickettsia*, *Ehrlichia* and *Anaplasma phagocytophilum* in small mammals and ticks from companion animals in Hualien, Taiwan

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Ticks are the primary ectoparasites of animals and serve as vectors for multiple bacteria, including *Anaplasma*, *Babesia*, *Ehrlichia*, and *Rickettsia* spp. which make threats to human health and animal health. Animals provide blood sources for ticks, becoming a reservoir for pathogens and may damaging either human or animal health. This study aim to determine the prevalence rate and identified species of Anaplasmataceae, and *Rickettsia* in ticks from both companion animals and small rodents and spleens from small mammals in Hualien, eastern Taiwan by PCR. In 2021 and 2023, a total number of 115 and 23 ticks were collected as samples from companion animals and small mammals, respectively. Moreover, a number of 41 small mammals including 8 *Apodemus agrarius*, 11 *Rattus losea*, 6 *Rattus norvegicus*, and 16 *Suncus murinus* were collected. Of the 115 ticks sourced from companion animals in 2021. The prevalence rate of Anaplasmataceae, and *Rickettsia* were 84.3% and 2.6%, respectively. Moreover, gene pairwise comparison suggested a putative new species, *Rickettsia* sp. HH-1, in *Haemaphysalis hystricis*. Of the 41 small mammals' spleens and 23 ticks collected in 2023, 0% (0/23) of ticks and 19.5% (8/41) of small mammals were infected with Anaplasmataceae. *Anaplasma phagocytophilum* and *Neoehrlichia mikurensis* were detected in 5 and 3 small mammals, respectively. The 16S rDNA amplicons showed 100% identical to *A. phagocytophilum* clone 10699S (Accession no.: MK394178.1) isolate from spleen in *R. losea* in Taiwan and 100% identical to *N. mikurensis* strain JXRLSY-59 (Accession no.: MH722225.1) isolate from spleen in *R. losea* in China. These results demonstrate that ticks and small mammals infected with *Rickettsia*, *Ehrlichia* and *Anaplasma* spp. are endemic to Taiwan. Under the One Health concept, the high prevalence of Anaplasmataceae and more species of pathogens indicating that owners in Hualien should pay more attention to ticks on animals and tick-borne diseases.

Keywords: Anaplasmataceae, Companion animals, One Health, *Rickettsia* spp., Small mammals, Tick.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

15:00-15:20

Molecular survey of vector-borne bacterial agents in lowland tapirs (*Tapirus terrestris*) from Brazil

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ABSTRACT

Although *Ehrlichia*, *Anaplasma*, *Borrelia* and *Bartonella* have been reported infecting animals worldwide, there are no reports on the occurrence of these vector-borne bacteria in lowland tapirs (*Tapirus terrestris*), the largest land mammals in Brazil. The present study aimed to molecularly detect these agents in blood samples from 99 wild tapirs from two different biomes in Brazil: Cerrado (CE n=38) and Pantanal (PA n=61). DNA from blood samples was extracted using a commercial kit and subjected to PCR for a mammalian endogenous gene (*gapdh*) to ensure DNA recovery. Positive samples were subjected to qPCR assays based on the *nuoG* and 16S rRNA genes of *Bartonella* and *Borrelia*, respectively, and conventional PCR assays targeting the *Ehrlichia dsb* gene and *Anaplasma* 16S rRNA gene. Positive samples were subjected to additional PCR assays targeting other molecular markers for additional molecular characterization, Sanger sequencing and phylogenetic assessment. All samples were negative for *Ehrlichia* sp., 8 (8.1%; 7CE, 1 PA) were positive for *Bartonella* sp., 2 (2%; 2 PA) for *Borrelia*, and 22 (22.2%; 7 CE, 15 PA) for *Anaplasma*. In the phylogenetic inference, eight tapir-associated *Anaplasma* 16S rRNA sequences (~1.2 Kb) clustered separately from other *Anaplasma* species, albeit closely to clades containing sequences of *Anaplasma odocoilei* and '*Candidatus Anaplasma capybara*'. The five tapir-associated *Bartonella ribC* sequences grouped with *Bartonella henselae*. One *Borrelia flaB* sequence obtained from a tapir grouped with *Borrelia theileri*. Two animals (2/99; 2%) from Cerrado biome were co-infected with *Anaplasma* and *Bartonella*. *Bartonella henselae*, *Borrelia theileri* and a putative novel genotype of *Anaplasma* sp. were detected for the first time in wild tapirs from Brazil. The impact of infections by these vector-borne bacteria in tapir health is unknown.

KeyWords: tapirs; *Anaplasma*; *Borrelia*; *Borrelia*; wildlife

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room Internacional I, Meliá Internacional Varadero

15:20-15:40

***Neoehrlichia mikurensis* in ticks parasitizing cave-dwelling bats**

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Bats serve as reservoirs for various viruses, bacteria, and protozoan parasites, of which many also infect humans. They harbor ectoparasites such as ticks, fleas, mites, and bat flies. *Neoehrlichia mikurensis* has been described in 2004 in Japan and recognized as a human pathogen in 2007. It is widely detected in both wild and domestic mammals and birds, and rodents appear to play an important role as reservoirs in the natural cycle of *N. mikurensis*. In our previous studies, three tick species, *Ixodes vespertilionis*, *Ixodes simplex*, and *Argas vespertilionis*, were identified as parasites in cave-dwelling bats in the Central Balkans. Regarding the occurrence of *N. mikurensis* in Serbia, its presence was recorded in *Ixodes ricinus* ticks collected from vegetation and in *Apodemus flavicollis* mice. The present study was initiated to determine the role of bats as potential reservoirs of tick-borne pathogens (TBPs). A total of 207 ticks collected from cave-dwelling bats in Serbia were analyzed for the presence of TBPs, and we're presenting the findings of *N. mikurensis*. The DNA samples of individual ticks were mixed into 21 pools, which were then analyzed by polymerase chain reaction. In case of positive pool, the individual samples in the pool were retested. The individual positive samples were first identified by sequencing 16S DNA fragment specific to the family Anaplasmataceae. In addition, the positive samples were analyzed by sequencing the partial *groEL* gene. In total, 11 samples were positive by PCR, while sequencing was successful in 7 samples. BLAST analysis revealed a high percentage of identity with previously published sequences of *N. mikurensis* available in GenBank. All ticks carrying *N. mikurensis* were identified as *I. simplex* and collected from *Miniopterus schreibersii* bats. To our knowledge, the present study provides the first detection ever of *N. mikurensis* in ticks collected from bats.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

15:40-16:00

A novel modeling tool for Rocky Mountain spotted fever intervention

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Laura Backus, School of Veterinary Medicine, Davis, USA

Patrick Foley, Biological Sciences, Sacramento, USA

Rocky Mountain spotted fever (RMSF) has emerged as an epidemic in western North America since before the turn of the 21st century. In this region, the brown dog tick, *Rhipicephalus sanguineus*, is responsible for spreading the disease between dogs and humans. The widespread nature of the disease and the ongoing epidemics contrast with historically sporadic patterns of the disease. Eliminating local epidemics with dog (eg neutering or population reduction campaigns) or aggressive acaricide treatment has rarely prevented epidemics. Because dogs act as reservoirs for the *Rickettsia rickettsii* bacteria, transmission dynamics between dogs and ticks are critical for understanding the epidemic. We present a compartment metapopulation model designed to explore the dynamics and drivers of RMSF in dogs and brown dog ticks in a theoretical region in western North America. We discovered an extended lag—as long as two years—between introduction of the pathogen to a naïve population and epidemic-level transmission, suggesting that infected ticks could disseminate extensively before disease is detected. A single large city-size population of dogs was sufficient to maintain the disease over a decade and serve as a source for disease in surrounding smaller towns. This novel tool can be used to identify high risk areas and key intervention points for epidemic RMSF spread by brown dog ticks.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

16:00-16:15

Tick-borne pathogens in Southern Norway: Seasonal variation of TBEV, *Borrelia burgdorferi* and *Neoehrlichia mikurensis*

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Ticks are highly dependent on biotic and abiotic factors for their reproduction and behavioral patterns as well as their survival and establishment. Climatic factors are involved in the interaction between the distribution and prevalence of ticks and tick-borne diseases, and macro- and micro-climatic conditions, will affect their questing behavior, molting, feeding and reproduction success and survival, and the behavior of their vertebrate hosts. *Borrelia burgdorferi* s.l. and *Neoehrlichia mikurensis* are the two most common pathogenic bacteria found in *Ixodes ricinus* in Norway, which can also carry the European subtype of the Tick-borne encephalitis virus (TBEV). *N. mikurensis* and TBEV have higher prevalences in ticks in the southern part of Norway than in the north, but the prevalence of *B. burgdorferi* is comparable between north and south. Surveillance of TBEV prevalence in ticks from Mandal (Vest Agder; near the south coast) has been carried out since 2009 until present. We have included monthly assessments of ticks and TBEV since 2017. The average prevalence was estimated to 0.35% in nymphs and 6% for adults with peaks in summer or early autumn months. *N. mikurensis* and *B. burgdorferi* s.l. were analyzed in nymphs from April to November in 2020, and this is the first time that the prevalence of these bacteria is analyzed monthly for an entire tick season in Norway. The overall prevalence was 12% for *B. burgdorferi* and 8% for *N. mikurensis*. The influence of relative humidity, temperature, precipitation, and saturation deficit, and hosts usage on tick density and the prevalence of these pathogens will be discussed.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

16:15-16:30

DIVERSITY AND MOLECULAR CHARACTERIZATION OF HEMOPLAMAS INFECTING BATS FROM THE AMAZON RAINFOREST REGIONS OF ACRE, BRAZIL

Isaac Leandro Lira Pinto¹; Eduarda de Oliveira Silva Lima Machado¹; Thiago Dutra Dias¹; Laís Feliciano de Souza¹; Tatiana Pádua Tavares de Freitas^{1,3}; Huarrisson Azevedo Santos²; Bernardo Rodrigues Teixeira³ & Maristela Peckle Peixoto¹

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This study sought to investigate the presence of hemoplasmas in bats sampled in the Amazon Rainforest regions of Rio Branco/Acre and contribute to their molecular understanding. Liver parenchyma samples from 111 bats collected in November 2021 had their DNA extracted using a commercial kit. A conventional PCR (cPCR) assay based on the endogenous *cytochrome B* gene was performed using primer L14724-CIT B - VER (1100pb) to confirm species identity. Furthermore, cPCR assays were performed using primers HemMycop16S-41s/HemMycop16S-938as and HemMycop16S-322s/HemMycop16S-1420as, which target 1300pb of the 16S *rDNA* gene of *Mycoplasma* spp. Samples were purified and then sequenced using the Sanger method. The assembled contigs were aligned with 30 *Mycoplasma* spp. sequences deposited in Genbank using the ClustalW method. The phylogenetic tree was constructed using the Maximum Likelihood method and Tamura-3-parameter+G evolutionary model based on an alignment of 918pb of the 16S *rDNA* gene of *Mycoplasma* spp. Positivity rate was 21.62% (24/111), obtained from the genera *Artibeus* 45.83% (n=11/24), *Carollia* 29.17% (n=7/24), *Desmodus* 4.17% (n=1/24), *Glossophaga* 4.17% (n=1/24), *Lophostoma* 4.17% (n=1/24), *Phyllostomus* 4.17% (n=1/24) and *Sturmira* 8.33% (n=2/24). Molecular characterization was obtained from 19 of the 24 positive samples. In the BLASTn analysis, the samples showed similarity ranging from 95.65% to 98.15% with *Mycoplasma* spp. sequences previously identified in hematophagous bats from Peru (KY932721) and Belize (KY932722). The samples were positioned within the "*hemofelis* group" revealing the presence of two well-differentiated groups of sequences, one composed of nine closely related sequences from the genus *Artibeus* and the other one from six bats of the genus *Carollia*. The other samples shared the same clade as hematophagous and non-hematophagous bats from Belize, Peru and Brazil. In conclusion, we report the existence of hemoplasmas infecting different bat species in the region studied and highlight the possible existence of two new genotypes of hemoplasmas parasitizing bats.



CUBA - 2024

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

16:30-16:45

Spanish Collaborative Project on Tick and Tick-Borne Pathogens Distribution (GARES)

María Vilá^{1,2}; María Sánchez²; Nélida Fernández³; Juan E. Uribe⁴; Agustín Estrada⁵; Miguel Ángel Habela⁶; Francisco Ruiz-Fons⁷; Jesús Barandika⁸; Lucía San Miguel⁹; Esteban Aznar⁹; Félix Valcárcel²; A. Sonia Olmeda¹

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Vector surveillance is essential to carry out prevention and control plans against vector-borne diseases. Aware of this necessity, the Spanish Ministry of Health (CCAES) promoted the GARES project (EU Next Generation) to complete the knowledge of tick distribution in Spain. Spanish tick experts have worked together with more than 230 citizen collaborators sampling questing and feeding ticks all over the nation. As a result, the most complete and updated tick distribution map has been created, collecting ticks from all counties allowing the access to remote areas and hosts.

Ticks from vegetation were collected in 325 sites while 635 animal collectivities (31 different species, including human) were sampled. A total of 10,500 specimens were identified from the subsequent species: *Dermacentor marginatus*, *Dermacentor reticulatus*, *Haemaphysalis concinna*, *Haemaphysalis hispanica*, *Haemaphysalis inermis*, *Haemaphysalis punctata*, *Haemaphysalis sulcata*, *Hyalomma dromedarii*, *Hyalomma lusitanicum*, *Hyalomma marginatum*, *Hyalomma scupense*, *Ixodes frontalis*, *Ixodes hexagonus*, *Ixodes ricinus*, *Ixodes ventralloi*, *Rhipicephalus bursa*, *Rhipicephalus pusillus*, *Rhipicephalus sanguineus* s. l.

- *H. lusitanicum* tick is the most abundant species in dry areas. Nonetheless, specific sites in the North with abundant populations were found. *H. scupense* and *H. dromedarii* are not well known species in Spain, however they were able to stablish permanently in certain spots.
- *Ixodes* ticks are limited to humid territories on the North of Spain, although small spots where certain conditions are reached also can harbor this species.
- *Rhipicephalus* genre has showed to be the most widespread one, being present depending on the hosts over environmental conditions.
- *Haemaphysalis* has the most biodiversity in the Iberian Peninsula, with 5 species identified.
- *Dermacentor* has been found in all the territory, but their populations are quite small.

Selected specimens were used for the detection of pathogens and phylogenetic studies through molecular biology techniques, allowing to reach a comprehensive understanding on Spanish ticks.

Acknowledgements: This investigation was made thanks to EU Next Generation, Spanish Health Minister and the Centro de Coordinación de Alertas y Emergencias Sanitarias (CCAES) of Madrid, PRAGMATICK cost action CA21170 and ESGARIBER (SOCEPA) group.

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room Internacional I, Meliá Internacional Varadero

16:45-17:00

Elimination of *Babesia ovis* from experimentally infected sheep: Significance of these animals for *Rhipicephalus bursa* larvae in a one-year season

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Keywords: *Babesia ovis*, experimental infection, *Rhipicephalus bursa*, sheep

Abstract

Babesia ovis, particularly transmitted by the vector *Rhipicephalus bursa* ticks in regions with high tick density, causes significant mortality rates in sheep, presenting symptoms such as fever, anemia, jaundice, and hemoglobinuria. Despite the importance of babesiosis in sheep, there is a lack of comprehensive research on the transmission patterns and factors impacting its epidemiology. In this study, we investigated whether sheep that survived babesiosis without treatment between May and July, when cases of the disease occur, could serve as a source of infection for sterile host-seeking *R. bursa* larvae in November-December. For this purpose, donor sheep (n=3) were experimentally infected with *B. ovis*/Alacakaya stabilate, and after six months, the elimination of *B. ovis* was assessed *in vivo* through blood and tick transmission experiments. For blood transfusion, 100 ml of blood was collected from the donor animals after six months and administered intravenously to recipient sheep (n=3). Simultaneously, 0.1 gr sterile *R. bursa* larvae were given to the donor sheep, and engorged nymphs were collected from these sheep. In the laboratory setting, engorged nymphs were permitted to complete their development into adult ticks. Subsequently, other recipient sheep (n=3) were each provided with batches of 50 female and 50 male *R. bursa*. Following blood and tick transmission, all recipient sheep (n=6) were monitored microscopically, serologically and molecularly for the presence of *B. ovis* for 100 days. The molecular and serological analysis confirmed the presence of *B. ovis* in three sheep that received blood, leading to clinical infection in two of them. Conversely, in the tick transmission experiment, three sheep were determined to be both molecularly and serologically negative for *B. ovis*. The blood transfusion experiment indicated the presence of the parasite in the donor animals, but the parasite load in the blood was insufficient to infect the larva-nymph stage. Consequently, experimental evidence reveals that sheep infected with *B. ovis* between May and July, recovering from the disease without treatment, do not serve as a source of infection for host-seeking *R. bursa* larvae in November and December.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Pathogenesis of ticks and tick-borne pathogens

Room Internacional II, Meliá Internacional Varadero

9:30-10:00

Proteases associated with the apical complex of *Babesia*

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Abstract:

Apicomplexan parasites use a cascade of specific proteolytic enzymes driving the function of the apical complex during egress and invasion of host cells. These processes are critical for propagation of *Babesia* through the asexual cycle in host erythrocytes causing for babesiosis. Analysis of *Babesia* omics datasets, leveraging knowledge from *Toxoplasma* and *Plasmodium*, identified two clade-C *Babesia divergens* aspartyl proteases, homologous to *Plasmodium falciparum* plasmepsins IX/X (*PfPMIX/X*) and *Toxoplasma gondii* *TgASP3*, designated *BdASP3a/b*. These are considered key drivers of apical complex processing associated with egress and invasion of host erythrocytes. Expression profiling across the *B. divergens* lifecycle revealed *BdASP3a/b* presence in blood stages but, in contrast with malarial *PfPMIX*, not in tick/vector stages. This indicates unique coevolutionary adaptations of piroplasms to the tick bloodfeeding behavior. *BdASP3s* were expressed as active enzymes in bacterial and baculovirus-infected insect cells. Immunomicroscopy utilizing gained polyclonal antibodies localized *BdASP3a* to apical complex associated organelles of intraerythrocytic *B. divergens*. Active recombinant *BdASP3s* were used to determine enzyme kinetics with *P. falciparum*-derived fluorescent substrates, and to confirm enzyme inhibition by 49C, the hydroxyethylamine inhibitor of *PfPMIX/IX*. Its addition to RBC-cultured *B. divergens* hinted *BdASP3a/b* roles in invasion rather than egress evidenced by the accumulation of free merozoites. Trans-genera complementation with iKD-*TgASP3* *T. gondii* strain suggested *BdASP3s*' involvement in protein maturation, mirroring *TgASP3*'s secretory pathway but not its deleterious phenotype. Importantly, our use of functional genomic tools -conventional and conditional gene KO/KD- is instrumental in unravelling the molecular phenotypes through proteomic analyses. These strategies are crucial to confirm the indispensable roles of *BdASP3a/b* and their validation as therapeutic targets. In conclusion, in line with *PfPMIX/IX*, the two *BdASP3* enzymes play analogous but not completely identical roles in *Babesia* propagation and represent druggable proteolytic targets for the development of specific chemotherapy.

Acknowledgements: This work was supported by projects 23-07850S and 21-11299S of the Czech Science Foundation (GAČR)

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

10:00-10:30

Advancing Visualization and Monitoring of Tick-Borne Encephalitis Virus Infection Using a Novel Reporter System

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Tick-borne encephalitis virus (TBEV) represents a pressing public health concern, necessitating novel insights into its infection dynamics, pathogenesis, and therapeutic strategies. Here, we introduce a novel approach how to study TBE pathogenesis and to screen for anti-TBEV drugs: the development and implementation of a recombinant reporter virus for TBEV. This innovation enables tracking of viral infection both in vitro and in vivo, shedding light on its intricate pathogenesis and facilitating the exploration of novel diagnostic and therapeutic avenues.

Our reporter system leverages TurboGFP (tGFP), a fluorescent protein expressed stably from the viral genome within infected cells. Through our experimentation, we validated the expression of tGFP in vitro and in vivo, affirming its utility as a robust marker for TBEV infection. Notably, our recombinant system exhibits exceptional stability, retaining reporter gene expression and fluorescent signal integrity across multiple passages.

Furthermore, to elucidate virus spread and tissue distribution within mouse brains, we devised a novel method to render brain tissue transparent while preserving the tGFP signal. Leveraging this approach, we achieved unprecedented 3D visualization of brain infection at cellular resolution, unraveling critical insights into TBEV neuropathogenesis.

In conclusion, our study introduces a highly stable and versatile recombinant system for monitoring TBEV infection dynamics, offering a valuable tool for high-throughput antiviral screening and facilitating comprehensive in vitro and in vivo investigations. This platform holds promise for advancing our understanding of TBEV pathogenesis and accelerating the development of effective countermeasures against this formidable viral threat.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Pathogenesis of ticks and tick-borne pathogens

Room Internacional II, Meliá Internacional Varadero

10:30-11:00

Exploring early Interactions of Lyme Disease Spirochetes with Cells in Skin: Insights from Volume Electron Microscopy

Vancová Marie^{1,2}, Martin Strnad^{1,2}, Jiří Týč¹, František Kitzberger^{1,2}, Jana Schrenková¹ and Ryan O. M. Rego^{1,2}

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The Lyme disease spirochete *Borrelia burgdorferi* is transmitted through the Ixodes ticks into the dermis of a mammalian host. Further dissemination to distal tissues occurs by hematogenous and non-hematogenous routes. However, the detailed mechanisms of dissemination and invasion are not well understood. Due to their slender morphology, spirochetes are difficult to visualize by standard light microscopy, therefore we employed advanced volume electron microscopy techniques, including serial block face scanning electron microscopy, micro array tomography, and transmission electron microscopy, to visualize the initial events occurring in mouse skin 1 hour post intravenous and/or subcutaneous injection of *B. burgdorferi sensu stricto* in the presence or absence of *Ixodes ricinus* ticks feeding in close proximity. Our goal is to elucidate in detail the complex interactions of *Borrelia* with skin components such as the extracellular matrix, immune cells, and particularly capillaries. Additionally, we investigate at high resolution and 3D the mechanisms of penetration of *B. burgdorferi* s.s., relapsing fever spirochetes, and the *B. burgdorferi* mutant in the Decorin binding protein (DbpA/B) across the endothelial cell barrier (human umbilical vein endothelial cells) in vitro, along with the immediate morphological response of the challenged cells.

This approach allows us to study in detail both the in vivo and in vitro responses to *Borrelia* infection, providing a deeper understanding of the pathogenesis of Lyme disease and potential therapeutic targets.

Acknowledgment: This work was supported by the GACR 22-18647K and Czech-Biolmaging large RI project (LM2023050 and OP VVV CZ.02.1.01/0.0/0.0/18_046/0016045 funded by MEYS CR)

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

11:30-12:00

Interactome and forces guiding barrier transmigration of Lyme disease *Borrelia*

Strnad, Martin^{1,2}, Kopecká, Jana¹, Týč, Jiří¹, Kitzberger, František¹, Oh, Yoo-Jin³, Hinterdorfer, Peter³, Hain, Lisa³, Vancová, Marie^{1,2}, Hejduk, Libor², Rathner, Adriana⁴, Rego, Ryan^{1,2}

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Systemic dissemination of microbial pathogens permits pathogens to spread from the initial site of infection to target tissues. A key step in dissemination is transmigration from vasculature into extravascular tissues and further across various mechanical barriers before reaching the target tissues, which is mediated by surface adhesion proteins. *B. burgdorferi* is a bacterial pathogen and the causative agent of Lyme disease in humans. After tick transmission, the spirochetes need to escape from the high immune pressure in the blood stream and traverse through the endothelial cell lining into extravascular tissues. On its way, *B. burgdorferi* is able to squeeze through dense, gel-like matrices and cellular junctions with pore sizes much smaller than the diameter of their body. Using atomic force microscopy-based single-molecule force spectroscopy, we have shown that this is achieved by forming transiently stable interactions with several protein components of the extracellular matrix. We have observed that a subset of borrelial surface proteins, particularly Decorin binding protein DbpA/B, can enhance the translational movement of spirochetes in the extracellular matrix of the host. Using nuclear magnetic resonance, we study in-detail the specific interactions of DbpA/B with GAG molecules present in the extracellular matrix of the host. To complement the mechanical studies of borrelial migration, we visualize *in-vitro* the transmigration of *Borrelia* through endothelial cells using advanced electron microscopy techniques such as serial block-face electron microscopy.

We acknowledge the GACR projects (22-18647K; 23-06525J) and BC CAS core facility LEM supported by MEYS CR (LM2023050 Czech-Biolmaging and OP VVV CZ.02.1.01/0.0/0.0/18_046/0016045).

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

12:00-12:15

**Vector capacity of *Rhipicephalus bursa* for the transmission
of *Babesia aktasi* n. sp. infecting for goats**

Mehmet Can Uluçeşme¹, Sezayi Özübek¹, Münir Aktaş¹

¹Department of Parasitology, Faculty of Veterinary Medicine, Fırat University, Elazığ 23200, Türkiye

Since the discovery of the first *Babesia* species in 1888, several species/subspecies/genotypes, including *Babesia aktasi* n. sp., have been described. Our recent survey revealed that *B. aktasi* n. sp. is highly prevalent (22.5%) in indigenous goats from Mediterranean region of Türkiye. The survey also revealed that *Rhipicephalus bursa* is the most common tick species in goats. This finding has raised the possibility that *R. bursa* could potentially serve as a vector for *B. aktasi* n. sp. To confirm this assumption, an experiment was conducted, and the vector capacity of *R. bursa* was evaluated. *Babesia aktasi* n. sp. stabilate with 10% parasitemia kept in the liquid nitrogen was thawed, and 15 ml was intravenously injected to an immune suppressed (splenectomy + dexamethasone) indigenous donor goat. Following the injection, the donor developed severe clinical babesiosis (fever, anemia, jaundice, hemoglobinuria), and high parasitemia (35%). Prior to infection, the donor was infested with the sterile laboratory colony of F2 *R. bursa* (16 females, 26 males, 2500 larvae). In the laboratory setting, unfed larvae from the engorged females and unfed adults from the engorged nymphs were obtained. The larvae hatched from the engorged females (n = 2100) were then feed on a rabbit and allowed to develop into unfed adults. Thus, the adult ticks presumed to be infected with *B. aktasi* n. sp. were obtained. Two immune suppressed recipient indigenous goats were infested with each 100 ticks (40 females, 60 males), and monitored for babesiosis. Additionally, 50 pools were created (each containing 3 ticks) and DNA was isolated. No infection was observed in the goats. Furthermore, PCR analysis of the isolated DNA did not yield any positive amplification products indicating the presence of the parasite. These findings demonstrated that *R. bursa* does not possess vector competence for the transmission of *B. aktasi* n. sp. This work was supported by funding from the Scientific and Technological Research Council of Türkiye (TUBITAK) (project no. 221O119).



CUBA - 2024

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

12:15-12:35

Co-infection dynamics of *Borrelia afzelii* and TBEV in C3H mice

Authors: Porcelli S, Heckmann A, Deshuillers PL, Galon C, Mateos-Hernández L, Rakotobe S, Simo L, Cabezas-Cruz A, Lagrée A-C, Moutailler S

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Ticks and tick-borne diseases (TBDs) are increasingly recognized as a critical One Health issue. In Europe, prominent TBDs include Lyme borreliosis, granulocytic anaplasmosis and tick-borne encephalitis (TBE), which are primarily transmitted by the widespread *Ixodes ricinus* tick. This vector has a wide geographical range and feeds on a variety of vertebrate hosts, potentially acquiring and transmitting multiple pathogens with each blood meal. TBDs are often associated with co-infections, where multiple pathogens coexist in a single host. For example, patients with chronic Lyme disease often have co-infections with other bacteria or parasites, highlighting the importance of understanding the outcomes of such interactions.

Our research aimed to develop a co-infection model of *Borrelia afzelii* (*B. afzelii*) and tick-borne encephalitis virus (TBEV) in C3H mice and to evaluate symptoms, mortality, pathogen invasiveness and fitness compared to single infections. Additionally, we investigated pathogen transmission from co-infected mice to uninfected ticks. We successfully established co-infection with *B. afzelii* and TBEV in C3H mice and observed different outcomes depending on the timing of co-infection. Specifically, delaying TBEV infection by nine days after *B. afzelii* resulted in exacerbated TBEV symptoms and increased viral load, whereas a twenty-one-day gap between infections resulted in milder symptoms and lower mortality. Simultaneous infection resulted in mild symptoms and no deaths. However, our model faced challenges in effectively infecting ticks with TBEV, possibly due to suboptimal dosing, highlighting the difficulty of replicating natural conditions. With the increasing prevalence of TBDs, understanding the impact of co-infection is crucial. Co-infected people can experience increased symptoms, highlighting the need for refined animal models to fully understand this phenomenon. Given the importance of C3H mice as a model for tick-borne diseases, further investigation of how the immune system responds to both pathogens will improve our understanding of the interplay between TBEV and *B. afzelii*.



CUBA - 2024

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Oral presentations

Pathogenesis of ticks and tick-borne pathogens

Room Internacional II, Meliá Internacional Varadero

12:35-12:55

Impairment of the host immune system impacts transmission and abundance of *Borrelia burgdorferi* in immature *Ixodes scapularis* ticks

Cody W. Koloski¹, Georgia Hurry¹, Alexandra Foley-Eby, Hesham Adam and Dr. Maarten J. Voordouw¹

¹ Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan

The process by which pathogens are transmitted from infected hosts to uninfected vectors is critical for understanding the epidemiology of vector-borne diseases. The efficiency of pathogen transmission depends on the abundance of the pathogen in the host tissues, which represents a balance between pathogen replication and the host immune response. The Lyme disease pathogen *Borrelia burgdorferi* (*Bb*) is a model system to test host-pathogen-vector interactions. In North America, *Bb* is transmitted to rodent reservoir hosts by the blacklegged tick (*Ixodes scapularis*). The spirochete establishes a chronic infection in the host tissues, but host-to-tick transmission often decreases over the course of the infection, suggesting the involvement of the acquired immune system. Mice with severe combined immunodeficiency (SCID) lack an adaptive immune response and often have a higher pathogen abundance compared to immunocompetent mice (IC). By manipulating the host pathogen abundance, we can determine if ticks acquire a *Bb* load proportional to the host *Bb* abundance. We can also track tick *Bb* abundance over the course of the host infection using multiple larval infestations, and over the course of tick development (fed larvae to nymph). We experimentally infected IC and SCID mice with *Bb* and subsequently measured pathogen transmission to ticks over the course of infection. We found that ticks that fed on SCID mice had a 4-fold greater *Bb* abundance than ticks that fed on IC mice. We also found that during tick development the pathogen abundance decreases in ticks that fed on SCID mice and increases in ticks that fed on IC mice. This research will enhance our understanding of how the host immune system influences the transmission of this important tick-borne pathogen and how the tick modulates the pathogen over the course of development. This research is funded by the Natural Sciences and Engineering Research Council of Canada.

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

12:55-13:15

Potential leucine aminopeptidase inhibitors of *Haemaphysalis longicornis* (HILAP)

B.S Susana Alberti Ramos, Center for Proteins Studies, Havana University, Havana, Cuba.
Ph.D Maikel Izquierdo Rivero, Center for Proteins Studies, Havana University, Havana, Cuba.
Ph.D Jorge González Bacerio, Center for Proteins Studies, Havana University, Havana, Cuba.

Ticks rely on blood as their primary source of sustenance, with hemoglobin being the predominant protein. Hemolysis is essential for them to obtain nutrients (Lejal et al., 2019).

In recent years, research has focused on characterizing the mechanisms of blood digestion in ticks. These studies have centered on individual proteases found in various species, representing different mechanistic classes (Karim et al., 2021 and Crispell G et al., 2019). Furthermore, research involving iRNA has demonstrated the significant role of leucine aminopeptidase from *Haemaphysalis longicornis* (HILAP) in decreasing fertilization (Hatta et al., 2006).

The importance of aminopeptidases has also been reported in the parasite *Plasmodium falciparum*, causative agent of malaria in which alanyl-aminopeptidase (PfA-M1) is present. In the parasite, the enzyme participates in the last stage of hemoglobin catabolism, being essential for its survival (Caperucci et al., 2010). In this sense, both aminopeptidases (HILAP and PfA-M1) participate in hemoglobin catabolism, which allows us to infer the possibility of targeting inhibitors with similar core structures. Several PfA-M1 inhibitors have been identified in our group, which could be potential tickicide.

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

13:15-13:30

Evaluating the Pathogenicity of *Babesia aktasi* n. sp. through experimental Infection in Saanen Goats and Sheep

Mehmet Can Uluçeşme¹, Sezayi Özübek¹, Münir Aktaş¹

¹Department of Parasitology, Faculty of Veterinary Medicine, Firat University, Elazig 23200, Türkiye

Babesia species, known for causing babesiosis in sheep and goats can lead to severe infections with high mortality rates in affected animals. Host breed plays a crucial role in the clinical outcome of babesiosis, although its impact on sheep and goats remains underexplored. Molecular parasitology advancements have revealed new species like *Babesia aktasi* n. sp., found in goats from Türkiye's Mediterranean region. In previous studies on the prevalence of *B. aktasi* n. sp. this was not found in sheep. In this study, it was aimed to evaluate the effects of *B. aktasi* n. sp. infection on Saanen goats and the infectivity and pathogenicity of the parasite in sheep. For this purpose, spleen intact (n=4), immunosuppressed (splenectomy + dexamethasone) (n=3) Saanen goats, and immunosuppressed sheep (n=5) groups were formed. These three groups were experimentally infected with fresh, *B. aktasi* n. sp. infected blood, and their clinical symptoms and hematological parameters were monitored throughout the infection. Piroplasm forms appeared in all goats one day post-inoculation. Both immunosuppressed and spleen-intact groups displayed severe symptoms, including fever, anemia, and hemoglobinuria. Rapid parasitemia increase in the immunosuppressed group led to mortality on the 4th or 5th day post-infection. The goats exhibited a significant increase in body temperature, with peak parasitemia reaching 33.5%. In the spleen-intact group, parasitemia rapidly increased to 7.6% on the third day post-infection, lasting 6-8 days, resulting in the death of two goats. In immunosuppressed sheep, specific clinical signs and babesiosis piroplasm forms were absent for one month post-inoculation. Nested PCR results showed two sheep positive for only one day, one sheep positive for four days, and two sheep negative. Hematological analysis showed significant decreases in RBC, HCT, and HB levels post-infection in splenectomized goats. Clinical symptoms and hematological parameters demonstrated that *B. aktasi* n. sp. is highly pathogenic to Saanen goats, leading to fatal infections. However, it was non-infective for lambs. This work was supported by funding from the Scientific and Technological Research Council of Türkiye (TUBITAK) (project no. 221O119).

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

9:30-10:00

Acaricide resistance: From genomics to a field-based genotyping assay

Michel Labuschagne

Clinglobal

Nanopore data, generated in a low resource environment, was used to generate, and annotate high quality *de novo* assembled genomes for *Rhipicephalus microplus* and *Rhipicephalus appendiculatus* ticks and their endosymbionts. The *R. microplus* genome was used to identify genes encoding acaricide targets as well as proteins involved in acaricide degradation. A novel Nanopore-based sequencing method was devised to sequence the full coding regions of 11 proteins involved in acaricide resistance from 37 geographically diverse isolates with phenotypic data for different acaricide classes. The deduced amino acid sequences were analyzed based on resistance factor score for the specific targets. Using the voltage gated sodium channel *kdr* mutation as an example, we designed a turnkey novel PCR field-based assay where the results are generated in less than 1 hour and fluorescence captured using a 3D-printed detection unit and a smartphone camera. The *kdr* ARDA (acaricide resistance detection assay) was highly specific and included an internal control to verify *R. microplus* identity. The novel PCR field-based assay can be universally applied and exhibits superior specificity based on a single nucleotide polymorphism. The 3D design files for the detection unit are freely available and the components readily available from most electronics/hobby stores. The complete detection unit can be 3D printed and assembled for less than US\$ 20 and the PCR based fluorescent assay allows for the easy conversion of standard PCR assays to a field-based genotyping assay.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

10:00-10:15

Identification of tick antigens after vaccination with extracellular vesicles in white-tailed deer

Julia Gonzalez¹, Cristina Harvey¹, Cárita de Souza Ribeiro-Silva², Brenda Leal-Galvan¹, Kelly A. Persinger³, Tammi L. Johnson³, and Adela Oliva Chavez^{1, 4}

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Current tick control measures are focused on the use of synthetic acaricides. Nevertheless, the emergence of acaricide resistance and the wildlife movements as a tick transport mechanism to introduce tick populations into new areas has prevented the efficient management of ticks. Thus, non-chemical control measures that can reliably reduce ticks within wild reservoirs are needed. This project aims to evaluate extracellular vesicles (EVs) from *Amblyomma americanum* vaccine candidates and identify antigenic proteins recognized by white-tailed deer (WTD, *Odocoileus virginianus*) immune responses. *Amblyomma americanum* female ticks were fed on 1-year-old WTD for 5 days on three different infestations. Salivary glands and midguts were cultured *ex vivo* in vesicle free tick media and extracellular vesicles were isolated by ultracentrifugation. Each WTD was vaccinated and received two boosters at 28 and 49 days. Two control deer were injected with adjuvant and PBS only. Reactivity of the serum was evaluated by western blot and ELISA. Serum from day 0 and 57 from one vaccinated and one control animal were used to immunoprecipitate antigenic proteins. At 58 days post vaccination/boost, WTD were infested with 100 *A. americanum* nymphs, 50 females, and 50 males that were allowed to feed to repletion. The western blot analysis using serum from vaccinated animals showed multiple bands in salivary EVs and midgut EVs not present in controls. Proteomic analysis of immunoprecipitated proteins identified 3 salivary EV and 6 midgut EV proteins found only in immunoprecipitations from vaccinated animals. No proteins were consistently precipitated in the serum from the control animal. Although not statistically significant there was a reduction in the number of females recovered from vaccinated animals. Further, early tick mortality during attachment was observed in one of the vaccinated animals. These results indicate that EV proteins from salivary and midgut *ex vivo* cultures can be used in the design of anti-tick vaccines.



CUBA - 2024

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

10:15-10:30

In silico identification and chemical remodelling of tick protein epitopes for vaccine antigen development

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Ticks and tick-borne diseases are severe burdens for healthcare systems and for animal husbandry amounting to billions of dollars in economic losses worldwide. Due to climate change, ticks' habitats are expanding, which makes the need for novel ways of tick control as pressing as never before. One of the desirable strategies is the development of anti-tick vaccines to elicit acquired tick resistance. The challenge in the development of such vaccines often lies in the inherently low immunogenicity of tick proteins, which they acquired through millions of years of evolution. Here we present a pipeline for the development of anti-tick vaccine antigens (ATVA) which utilizes AlphaFold2 structure modelling of tick proteins, *in silico* identification of antigenic epitopes by protrusion-based algorithms, their chemical remodeling and multimerization. The pipeline was applied to the tick salivary lectin pathway inhibitor (TSLPI) from *I. scapularis* as it plays a crucial role in *B. burgdorferi* transmission. Bioinformatic analysis showed the presence of a 10-residue b-hairpin designated as an antigenic epitope. This peptide was synthesized using solid-phase peptide synthesis and cyclized to preserve its secondary structure as in the parent protein, which was confirmed by NMR and CD spectroscopy. Further, the cyclized epitope was tetramerized using the chemically synthesized lysine wedge. Both monomeric and tetrameric epitopes were coupled to the carrier protein KLH and used in mice immunization experiments. ELISA analysis showed that the tetrameric epitope caused a 100-fold higher titer of TSLPI-specific antibodies than the monomeric construct. Then, rabbits were immunized by the TSLPI or tetrameric TSLPI epitope and challenged by ticks. Animals immunized by epitopes showed a higher level of TSLPI-specific antibodies, lower tick weights and egg hatching rate compared with the control and TSLPI. Finally, the applicability of a proposed pipeline on the tick transcriptome level was explored revealing dozens of potential new candidates for ATVA.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

10:30-10:45

The Translationally Controlled Tumor Protein (TCTP) of *Babesia bovis* induces neutralizing antibodies and participates in the establishment of an acute infection

Authors

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4. National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Japan.

Babesia bovis is a protozoan causing bovine babesiosis. It has been postulated that, in Apicomplexa parasites, the Translationally Controlled Tumor Protein (TCTP) interferes with the immune response, by blocking the interaction of host TCTP with its receptor, preventing the activation and proliferation of B-cell lymphocytes. The aim of this work was to characterize *B. bovis* TCTP activity as an immunogen against an acute *B. bovis* infection. In the present project, the complete *tctp* gene was amplified and sequenced in *B. bovis* isolates; the gene and the predicted protein sequences were highly conserved. Transcription was confirmed by RT-PCR and expression was also confirmed by WB and confocal microscopy. By bioinformatics tools the predicted three-dimensional structure was obtained, showing the characteristic alpha helix. Peptides containing predicted B-cell epitopes were designed, synthesized, and used in immunization assays, demonstrating their immunogenic capacity by inducing specific antibodies. Cattle were immunized with a mix of TCTP peptides then, they were challenged with a virulent strain of *B. bovis*. Clinical signs and parasitemia were monitored for 15 days. Less severe clinical signs were observed in immunized animals compared with controls. A lower amount of total antibodies ($p > 0.5$) was observed in the serum of animals in the control group after challenge, indicating an interference of *B. bovis* TCTP in the bovine immune response. A neutralization assay was carried out using *in vitro* cultured *B. bovis*, a percentage inhibition of 32-34% was observed using sera from immunized cattle. These results indicate that *B. bovis* has a *tctp* gene that is transcribed and expressed in intraerythrocytic stages. The protein contains peptides with conserved B-cell epitopes, which induce neutralizing antibodies in immunized animals. Anti-TCTP antibodies help reduce the clinical signs and improve the humoral immune response of infected cattle. Funded by UAQ-FONDEC (FNV-2020-06), USDA-ARS (59-2090-1-001-F), OUAVM and The Japan Society for the Promotion of Science.



CUBA - 2024

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

10:45-11:00

NEXT GENERATION TICK & TICK-BORNE DISEASE CONTROL STRATEGIES IN UGANDA: PREPARING FOR THE POST-ACARICIDE ERA!

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Abstract

Millions of metric tons of acaricides have been used especially in the tropics to kill and possibly eliminate ticks. However, ticks are still here with us and have adopted advanced survival strategies such as resisting major acaricides which used to kill them. In addition, the tick acaricide resistance challenge has become widely distributed in all regions of Uganda complicating options for acaricide zoning. Furthermore, the rate of new acaricide product innovations has not in any way matched the rate of tick resistance development leaving farmers with limited options. In addition, we continue to witness an upsurge in tick-borne diseases (TBDs) incidence at farm level leading to unprecedented cattle morbidity, mortality and increased production costs. In an attempt to fight against acaricide resistance, farmers reported using dangerous chemicals and concoctions including use of crop pesticides and herbicides for tick control. Therefore, we postulate that the era of relying on acaricides for tick and TBDs control could soon come to an end and therefore in this paper we propose the next generation strategies for sustainable control of ticks and TBDs in a post acaricide era. Broadly, the strategies include developing and adopting vector infection control technologies (VICT), strategic chemotherapy (Triple Combo), modifying the host and environment plus shifting efforts from tick control to TBDs control at reservoir/host level. The above strategies could ensure that animals and ticks co-exist in one ecosystem yet causing limited to no harm to each other and the environment leading to sustainable TTBDs control.

Key words:

Ticks, Tick-borne diseases, Acaricide resistance, Vector infection control technologies, Triple combo, and Post-acaricide era



CUBA - 2024

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Oral presentations

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

11:30-11:45

The Tick Cell Biobank – fifteen years of this unique research resource

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Abstract

The Tick Cell Biobank (TCB) was founded fifteen years ago at the University of Edinburgh; after a stint at The Pirbright Institute we moved to our current, permanent home at the University of Liverpool. Since 2009, we have distributed cell lines derived from ticks and other arthropods to over 100 institutes in Europe, Africa, Asia, North and South America and Australia. During this time, we and our Outposts in Asia (Malaysia), Africa (Kenya) and South America (Brazil) have trained over 170 scientists from 39 countries in generation and/or maintenance of tick and other arthropod cell lines. We can now provide training not only in person but also via remote live streaming which greatly facilitates dissemination of expertise and practical demonstration of cell lines and associated techniques.

The TCB now houses over 70 cell lines derived from 20 ixodid and three argasid tick species, as well as a smaller number of cell lines derived from sand flies, biting midges, mosquitoes, triatomine bugs, tsetse flies and honeybees. We are currently working on generation of new embryo-derived cell lines from *Dermacentor reticulatus*, *Hyalomma marginatum*, *Rhipicephalus bursa*, *Rhipicephalus microplus* and *Argas reflexus*. The TCB also houses a small collection of intracellular arthropod-borne bacterial pathogens and endosymbionts of the genera *Anaplasma*, *Ehrlichia*, *Rickettsia*, *Spiroplasma* and *Wolbachia*. We are developing a range of genomic and transcriptomic resources to accompany the tick cell lines including several mitogenome sequences.

The TCB Outposts also house and supply a range of the most popular and regionally relevant cell lines in support of locally based tick and tick-borne disease research; of interest for TTP11 delegates, the South America Outpost at the Oswaldo Cruz Institute in Rio de Janeiro is run jointly by Fiocruz and Universidade Federal Rural do Rio de Janeiro. For further information, contact tickcellbiobankenquiries@liverpool.ac.uk or visit our website at <https://www.liverpool.ac.uk/research/facilities/tick-cell-biobank/>



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

11:45-12:00

Unlocking Bacteriophages: Pioneering Diagnostics and Marker Discovery for early diagnosis of Lyme and tick-borne diseases

Jinyu Shan, PhD

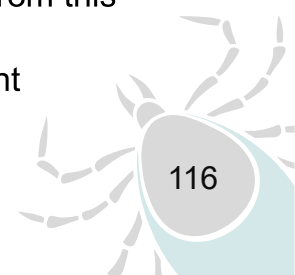
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Abstract

Bacteriophages, viruses that specifically infect bacteria, can exist as lytic phages or present within bacterial genome as prophages. These phages hold considerable promise for exploitation as biomarkers for the accurate and sensitive detection of bacterial infections. This assertion rests upon two foundational scientific insights: Firstly, the widespread presence of prophages within bacterial genomes—research indicates up to 90% of bacteria may harbour prophages—enables these elements to act as dependable indicators for identifying specific bacterial species. Secondly, the fact that a significant proportion of bacteria possess multiple copies of prophages enhances the sensitivity of targeting these prophages over single-copy bacterial genomes for detection purposes. Moreover, prophages have the capability to spontaneously exit their bacterial hosts and enter the circulatory system, rendering them more readily detectable compared to their often-elusive bacterial hosts and thus sometimes making direct bacterial detection unfeasible. Drawing from our work with Lyme disease phages, we've observed that phages associated with Lyme disease are tenfold more abundant than the bacteria causing the disease. This breakthrough heralds the beginning of a novel approach in tackling tick-borne illnesses and bacterial infections at large, marking a pivotal shift in global bacterial detection methodologies. Our prototype demonstrates our capacity to equip healthcare professionals with an essential diagnostic tool, signifying a revolution in medical diagnostics.

Ongoing refinements and enhancements to this technique are expected to broaden its applicability, thereby increasing the number of patients who could benefit from this cutting-edge diagnostic approach. Such advancements are crucial in the management and treatment of tick-borne diseases, highlighting the paramount importance of innovative diagnostics in revolutionising patient care.



Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

12:00-12:15

Acaricide resistance: The performance of acaricides active ingredients and acaricide formulations against tick treatments in bioassay tests

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Abstract

In the history of tick control, acaricides have played a key role to reduce ticks loads by breaking the lifecycle of ticks through different action modes: i) killing ticks, ii) inhibiting oviposition of eggs and iii) reducing the hatching ability of laid eggs. Recent field studies in Laikipia revealed that cattle owners expressed concern about perceived tick resistance, and adapted their practices to treating ticks. Whether these adapted practices yielded better results ticks remains to be tested. We seek to answer, i) What is the level of acaricide resistance and how do livestock owners deal with it? and ii) What is the performance of different acaricides' active ingredients and acaricide formulations used by cattle owners in Laikipia county, Kenya?

A larval packet test (LPT) was used to test the performance of four active ingredients (amitraz, chlorpyrifos, cypermethrin and combination of cypermethrin and chlorpyrifos) using technical grade formulations by quantifying the percentage larvae kill at different concentrations. An adult immersion (AIT) tested the mortality rate, oviposition rate and hatchability of larvae from eggs treated with acaricides at the recommended dilution ratios, and farmers' mixtures of acaricides.

From LPT results, chlorpyrifos gave 100% mortality at 0.1 concentration at the expected DS of 0.1. Combination of chlorpyrifos and cypermethrin gave 100% mortality at the highest concentration of 0.8 against a 0.2 DS concentration. Low mortalities were observed with Cypermethrin (50%) and Amitraz (38%) at 0.8 concentration. In the AIT, combination acaricide gave 100% mortality after 14 days and inhibited eggs to oviposition. Tick mortality rates decreased progressively from Cypermethrin, to Alpha-cypermethrin, Amitraz acaricides having the lowest mortality. Both cypermethrin and Alpha-cypermethrin had low oviposition rates however the eggs hatched to larvae while Amitraz had a high oviposition rate with low percentage of larvae hatched.

These results give indication of resistance to Amitraz and synthetic pyrethroids.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

12:15-12:30

Changes in the serum proteome profile of patients with neuroborreliosis, foresters, and patients treated according to ILADS method

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Lyme borreliosis (LB) is the most frequent tick-borne disease. The inappropriate diagnosis of LB leads to therapeutic wandering. Early and proper detection of LB is essential to propose an adequate treatment.

To evaluate the changes in proteomic profile of LB serum patients. We aimed to compare neuroborreliosis (NB) patients with patients, who were treated with prolonged multidrug therapy according (ILADS method) and foresters who were frequently exposed to tick but didn't have any characteristic symptoms.

Blood was collected from 37 patients divided into the groups:

Group I – 10 patients with NB

Group II – 10 non-hospitalized foresters

Group III – 7 patients who were treated according to ILADS method

Group IV – 10 control group

High-performance liquid chromatography system (Ultimate 3000; Dionex, Idstein, Germany) and exactive HF mass spectrometer with an electrospray ionization source (Thermo Fisher Scientific, Bremen, Germany) were used for eluted peptides analyzing.

Obtained results indicated significant differences in the proteomic serum profile of NB patients, patients treated according to ILADS method and foresters comparing to Ctrs. Proteomic approach allowed to identify and quantify the expression of 293 proteins, within 122 were significantly changed. The principal component 1 (PC1, 30.8%) significantly separated Ctr, ILADS and NB groups, while PC2 (6.3%) particularly strongly filtered out Ctr. Only Foresters were not clearly grouped.

Obtained results indicating changes in the serum proteome of NB patients put attention to the proteins involved in calcium transport/metabolism, and signaling molecules that differ patients before and after EBM therapy. Patients treated with ILADS method have different protein distribution than other patients, what does not support the view that ILADS patients reflects an ongoing *Borrelia burgdorferi* infection. β -secretase 2 found in foresters without any symptoms, may indicate a soon-developing neurodegenerative disease as a result of frequent tick-borne infections.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

12:30-12:45

**Exploration of emerging tick-borne pathogens and neglected tick-borne diseases in Serbia:
Balkan model of One Health approach**

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Ticks (order Ixodida) are obligate blood-feeding ectoparasites of mammals, birds, and reptiles, which are globally important vectors of disease-causing agents that impact both human and animal health. Here we present the results of "One Health" approach implemented as interdisciplinary method to study the spread of tick borne pathogens (TBPs) between ticks, animals and humans, as well as integration of xenodiagnostic procedures in healthcare management of humans with tick infestation.

Throughout 4 consecutive years (2019-2022) we conducted a series of studies under "One Health" approach which included enrollment of patients and tick removed from them, as well as investigation of possible TBP foci within household of patients with emerging TBDs. Materials and datasets generated through this approach were analyzed via entomologic, microbiological and molecular analysis, including microfluidic real-time high-throughput PCR system that was used to test the DNA of the tick and human blood for the presence of 27 bacterial and eight parasitic TBPs.

From all ticks removed from humans *Ixodes ricinus* was the most frequent tick species. Different *Rickettsia* species were the most common TBPs identified in the ticks. We have detected and described cases of atypical infections caused by *R. helvetica* and *R. raoultii*, exposure to Tick-Borne Encephalitis virus as well as fatal imported case of Tick-Borne Encephalitis. In addition, we examined the platelet fraction from patient blood as a substrate for direct detection of TBPs causing bacteremia. One Health approach described in Serbia (Balkan model) is first implemented worldwide and will help to characterize the components of the chain of infection leading to human infection by TBPs as well as diagnostics of tick borne diseases caused by emerging pathogens.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

12:45-13:00

**Association of the bovine leukocyte antigen major histocompatibility complex exon II
DRB3*020:02:01 to host resistance to *Theileria orientalis* infection in crossbred Kedah-
Kelantan cattle**

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Abstract

The bovine leukocyte antigen (BoLA) is used as a disease marker due to its major genetic role in pathogen recognition and presentation by the adaptive immune system. In this study, the major piroplasm surface protein gene of *Theileria orientalis* was detected in 37 out of 85 (43.5%) crossbred Kedah-Kelantan cattle. The allelic association of the *BoLA-DRB3* gene with resistance and susceptibility to *T. orientalis* infection was evaluated in the affected cattle. The association between an allele and *T. orientalis* were evaluated by Fisher's exact and Cochran Mantel Haenszel (CMH) test. The odds ratios (OR) and their 95% confidence intervals for susceptibility or resistance were calculated for each allele. The Bonferroni correction procedure was performed to adjust the false-positive rate. Association tests having a significance level of $p < 0.0125$ (corrected p -value) were considered statistically significant. The PCR-sequence based typing of *BoLA-DRB3.2* gene from KKB revealed that the gene is highly polymorphic. Ten novel alleles were detected (*BoLA-DRB3*012:04*, **015:08*, **015:09*, **015:11*, **015:12*, **017:05*, **017:07*, **024:33*, **107:04*, **168:01*) in KKB cattle, and these alleles shared about 90.7-95.8% and 85-92% nucleotide and amino acid identities respectively, with the *BoLA-DRB3*016:01* cDNA clone NR-1. Eighteen alleles were identified in the *T. orientalis* infected cattle namely *DRB3.2*007:01*, **007:05*, **009:02*, **011:01*, **012:01*, **012:04*, **015:01*, **015:12*, **016:01*, **017:01*, **018:01*, **027:03*, **036:01*, **057:02*, **020:02:01*, **050:01:01*, **026:01* and **041:01*. In KKB cattle. The associated allele of *T. orientalis* infection susceptibility in the KKB was *DRB3*017:01* (OR = 5.667; P_{CMH} = 0.004) while resistance allele was *DRB3*020:02:01* (OR = 0.091; P_{CMH} = 0.003). Therefore, this study identified *BoLA-DRB3.2* alleles associated with resistance and susceptibility to *Theileria orientalis* infection and suggests that during breeding, genetic selection of resistant animals could be a natural strategy for control of Theileriosis, particularly when there is no available global vaccine for its prevention.

Keywords: bovine leukocyte antigen; alleles; *Theileria orientalis*; Kedah-Kelantan x Brahman cattle; PCR-Sequence based typing.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

13:00-13:15

Effects of Ash on *Ixodes pacificus* and *Dermacentor Spp.* Survival, Behavior, and Host Interaction

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Introduction

Dustbathing is common among animals, including those that serve as disease hosts, and is posited to help reduce ectoparasite burden. People have attempted to control arthropod pests by using ash on domestic animals since at least the 2nd millennium BCE. However, effects of bathing in ash on the burden levels of ectoparasites that transmit zoonotic disease has yet to be quantified. However, the precise impact of ash bathing on ectoparasite levels responsible for transmitting zoonotic diseases remains unquantified. Furthermore, the influence of ash on ticks and tick-borne disease dynamics within fire-affected ecosystems has yet to be explored.

Methods

Ash was generated from *Sequoia*, *Quercus*, *Laurus*, and *Eucalyptus* trees and compared to diatomaceous earth, kaolin clay, and no exposure as controls. To mimic the effects of dustbathing on the vector, *Ixodes pacificus*, *Dermacentor albipictus*, *D. occidentalis*, and *D. similis* larvae and adults were rolled in their respective exposure material. Immediately post-exposure, ticks were placed upside down and the time until they righted themselves measured to assess disorientation. Ticks also were maintained at RH> 80%, 70° C and assessed for survival post-treatment. Ticks were then exposed to mice to assess whether ash exposure on the host influences burden levels.

Results and Discussion

Ash produced from *Laurus* and *Eucalyptus* had effects on righting and survival that rival the effects of diatomaceous earth and kaolin clay on mortality and disorientation. These findings have implications on post-fire tick-borne disease dynamics and suggest that plant community structure may impact tick and tickborne disease dynamics during prescribed burns and wildfires due to the ash produced.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

13:15-13:30

Screening for immune biomarkers associated with infection or protection against *Ehrlichia ruminantium* by RNA-sequencing analysis

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Heartwater is one of the most economically important tick-borne fatal diseases of livestock. The disease is caused by the bacteria *Ehrlichia ruminantium* transmitted by Amblyomma ticks. Although there is evidence that interferon-gamma controls *E. ruminantium* growth and that cellular immune responses are protective, an effective recombinant vaccine for this disease is lacking. Analyses of markers associated with infection as well as protection will lead to a better understanding of the *E. ruminantium* immune response and corresponding pathways induced in sheep peripheral blood mononuclear cells (PBMC) will assist in development of such a vaccine. In this study, Biomarkers of infection (BMI) were identified as uniquely expressed genes during primary infection and biomarkers of protection (BMP) associated with immune to heartwater were identified post challenge. Sheep were experimentally infected and challenged with *E. ruminantium* infected ticks. The immune phenotypic and transcriptome profile of their PBMC were compared to their own naïve PBMC collected before infection. The study revealed 305 differentially expressed genes (DEGs) as BMI, of these 17 were upregulated at all three time-points investigated. These DEGs, form part of the bacterial invasion of epithelial cells Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway, and others detected from day 1 post infection and are considered predictive markers for early heartwater infection in ruminants. Similarly, a total of 332 DEGs were identified as BMP, of these 100 were upregulated and 75 were downregulated at all three time-points investigated. However, at D1PC most DEGs were downregulated (n = 1312) that correlated with a reduction in the % CD4 and CD8 T cells detected with flow cytometry. KEGG pathway analyses showed complete down regulation of T cell specific pathways possibly due to homing of immune cells to the site of infection after acquired immunity developed. At D4PC, expression levels of most of these downregulated genes increased and by D6PC they were upregulated. This indicates that the sampling time-point for biomarker analyses is important when results for acquired immune responses are inferred. This data identified DEGs that could be considered as biomarkers of protective immunity that can be used for identification of vaccine antigens and provides a strong foundation to further development of heartwater recombinant vaccines.

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

15:00-15:25

Evaluation of the Effectiveness of the GAVAC® Vaccine and Rational Use of Acaricides as an Alternative in an Integrated Tick Control Program in Ecuador.

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ABSTRACT

Ecuador is a tropical country located in South America, facing significant challenges in livestock management due to the prevalence of the *Rhipicephalus microplus* tick. This ectoparasite is recognized for its considerable sanitary and economic impact on Ecuadorian and global livestock industries. Current evidence indicates that chemical control methods are ineffective and contribute to the development of acaricide resistance, escalating costs and health issues. The indiscriminate and combined use of these chemicals has also promoted resistance development in ticks. Research has shown that the technical and combined use of two or more control alternatives can significantly reduce tick populations. One of these alternatives include the administration of a vaccine. This study evaluated the effectiveness of immunization with Gavac® vaccine and its impact on rational acaricide use, assessing the immunization's effect on the biotic potential of *Rhipicephalus microplus*, infestation levels, and the frequency of acaricide treatments. In this study, 176 animals were vaccinated following the recommended vaccination schedule: an initial dose, a booster after 30 days, and a second booster after six months. Among these, 30 animals were monitored every 15 days over eight months to evaluate the vaccine's effect on the ticks' reproductive potential. A significant reduction in tick burden on the animals was observed, alongside a decrease in chemical treatment usage by less than 10% (one treatment). The average interval per acaricide treatment extended to 23 days post-vaccination. A statistically significant reduction in the number of ticks on animals, the weight of the eggs, and their hatching rate was observed following the program's implementation. However, no significant effect was noted on the weight of engorged ticks. In conclusion, the vaccine demonstrably impacts tick presence on animals and on their reproductive aspects. Nonetheless, further immunological studies are required to substantiate these findings.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

15:25-15:50

Current status of vaccination against the cattle tick *Rhipicephalus microplus* in Mexico

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The cattle tick *Rhipicephalus microplus* is the most important ectoparasite affecting the livestock industry worldwide. This tick is a vector of bovine babesiosis and anaplasmosis, the most important tick-borne diseases that negatively impact livestock in tropical areas due to high morbidity and mortality in naïve cattle. The intensive use of chemical acaricides and poor management have contributed to genetical selection of resistant tick populations. Currently, resistance to all acaricide groups has been documented. Therefore, anti-tick vaccines represent a feasible and environmentally friendly alternative for tick control to mitigate tick resistance. In Mexico, vaccination against *R. microplus* started in the 90s, when the Bm86-based GavacTM was registered and commercialized by Revetmex, which distributed the vaccine until 2014. Lapisa, a Mexican pharmaceutical company which both, tick control products and technical services are part of the company's portfolio, released Bovimune IxovacTM, a tick vaccine based on Bm86 from a Mexican isolate. Currently, 470,990 cattle in the country have been vaccinated with this vaccine. The main effects to this vaccine in the field include a decrease on tick feeding, tick survival, oviposition, and fertility. However, the most important effect is the reduction of acaricide treatments, which ranges between 44 and 80%. Bovimune IxovacTM in combination with acaricides in programs designed for specific ranches, has demonstrated to be useful to control *R. microplus*. However, improved vaccines targeting various *R. microplus* isolates, including *Amblyomma mixtum*, are needed. Tick control using tick vaccines in areas with multi-tick resistance highlight the need for effective broad-spectrum tick vaccines against *R. microplus* and *Amblyomma* spp. that co-infest cattle.

Key words: Anti-tick vaccines, *Rhipicephalus microplus*, *Amblyomma mixtum*, resistance, integrated control

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

15:50-16:15

Immunization of cattle with *Rhipicephalus microplus* voraxin peptides, which contain predicted B-cell epitopes, decreases tick fitness.

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Abstract

Rhipicephalus microplus is the most widely distributed tick worldwide and causes significant economic losses in the livestock industry. It directly affects cattle by feeding on blood and damaging the skin, and indirectly acting as a vector of pathogens that cause infectious diseases, such as bovine babesiosis. Vaccines offer an ecologically friendly alternative approach to tick control. To develop novel tick vaccines, it is crucial to identify and evaluate antigens capable of generating protection in cattle. Voraxin is a protein necessary to stimulate the repletion of females after copulation, increasing its weight beyond a critical limit necessary for females to oviposit. In this study, the *R. microplus* voraxin gene was identified and its capacity to reduce fitness in ticks fed on immunized cattle was evaluated. The predicted amino acid sequence was obtained from five isolates and analyzed by informatics to identify four conserved peptides containing predicted B-cell epitopes. The immunogenicity of each peptide was assessed by inoculating two cattle, four times, and the antibody response was assessed by indirect ELISA. A challenge experiment was conducted with those peptides that were immunogenic. The voraxin gene was amplified, sequenced, and were compared with reference strains. A 98.69% identity and 96.73% similarity were obtained. Efficacy percentages of 51 and 27 were obtained with voraxin 3, and voraxin 4. This is the first report of a reduction in fitness in *Rhipicephalus microplus* ticks using voraxin as antigen.

Keywords: *Rhipicephalus microplus*, voraxin, tick vaccines, immunization, B-cell epitope.

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

16:15-16:40

Anti-tick vaccines and commercial challenges

Ala E Tabor^{1,2} and José de la Fuente^{3,4}

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The control of ticks continues to rely on preventatives and chemicals with poor adoption of novel tick vaccines. In terms of arthropod ectoparasite vaccines, research into tick vaccines has advanced but commercialisation success has been limited to Bm86 vaccines in the early 1990s. Alternatives to Bm86 have not progressed and currently GAVAC is still in production in Cuba, but TickGARD was discontinued in Australia. The global pesticide market is large with a recent trend to produce generic products which can protect hosts from fleas, lice, mites, flies, and ticks. In addition, these chemicals require regular administration which is more profitable than annually boosted vaccines. With a focus on the *Rhipicephalus microplus* species complex which transmits babesiosis and anaplasmosis to cattle in tropical and sub-tropical regions of the world costing ~\$US22-30b losses per annum, many new candidates have been patented and or published. For example, Subolesin tick protective antigen is currently under evaluation for vaccine approval in Uganda for control of cattle tick infestations. In Australia, two candidates have demonstrated 90% efficacy following two artificial larval challenges and are currently under further testing. Although results using Bm86 alternative vaccines are excellent, pharma claim that they desire vaccines that achieve the same larval and/or adult tick knockdown as observed when using acaricides. There is also evidence that regulatory bodies in certain jurisdictions require this efficacy to register new anti-cattle tick vaccines. Alternatives to these challenges are the application of “personalized medicine” considering local tick/host/pathogen genetic factors for the design of tick protective antigens and the rational combination of tick vaccines with bioacaricides and other control interventions. Furthermore, pharma’s dream is that one vaccine candidate could protect against all cattle tick species globally is the ‘holy grail’ however, until all representative genomes are sequenced achieving this goal is an unknown possibility.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

16:40-17:00

Change of mentality when it comes to anti-tick vaccine commercialization

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Chemicals continue to be the main method used for tick control. However, their use requires continuous treatments because there are no effects on tick populations. This intensive use produces food contamination, environmental pollution and the generation of chemical resistant tick strains. In this scenario, vaccination constitutes an environmentally friendly alternative with effects on the tick population control and that should be effective against all acaricide resistant tick genotypes. However, only few anti-tick vaccines have been commercialized which is a general rule for all parasitic diseases. There are few available vaccines against parasites and most of them show partial or limited protection. This is because parasites are scientifically challenging. Even the simplest parasites, have highly complex life cycles and are also biological and biochemistry complex. They have also developed ability to evade the host immune system and our understanding of the host/parasite interaction is still scarce. On the other hand, developing vaccines against parasites is also economically challenging because the vaccine efficacy is compared to that of chemical products. Besides, there is no strong market incentives for these vaccines because parasitic infections mainly impact poor people in regions of low economic power. In this scenario, a change in our minds is necessary to understand that a single method will not be effective enough to achieve the control of tick infestations. It is necessary the harmonious integration of various methods where vaccines could be applied as a backbone in an integral management strategy in which chemicals will be also included.



CUBA - 2024

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

9:30-10:00

The *Rhipicephalus sanguineus* group taxonomy: unclosed gaps

Filipe Dantas-Torres

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The *Rhipicephalus sanguineus* group includes a series of tick species morphologically and phylogenetically related to *Rhipicephalus sanguineus* (*sensu stricto*), a species originally described back in 1806. The taxonomy of this species remained a subject of debate for over two centuries. In 2018, this species was finally redescribed and a neotype designated, based on tick specimens collected from France. This important taxonomic act bridged the major gap in the taxonomy of the *R. sanguineus* group, facilitating subsequent acts, such as the revalidation of former synonyms (e.g., *Rhipicephalus linnaei*, *Rhipicephalus rutilus*, and *Rhipicephalus secundus*) and the description of a new species (*Rhipicephalus afranicus*). These and other studies published during the past two decades, allowed a comprehensive analysis of the *R. sanguineus* group, providing us with new insights into the geographical distribution and vector competence of *R. sanguineus* and related tick species. Under the frame of the 11th Ticks & Tick-borne Pathogen Conference, I had the pleasure to chair the special symposium on tick taxonomy and to deliver this talk on unclosed gaps in the taxonomy, geographical distribution and vector competence of tick species belonging to the *R. sanguineus* group.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

10:00-10:20

Perspectives on Argasid Evolution

Ben J. Mans

Epidemiology, Parasites and Vectors, Agricultural Research Council – Onderstepoort Veterinary Research, Onderstepoort, South Africa

Ticks (Ixodida) are composed of three extant families, the Argasidae, Ixodidae and Nuttalliellidae. The Argasidae was classified into two sub-families, the Argasinae and Ornithodorinae based on morphological characters. Molecular systematics also differentiate two major clades that correspond in broad terms to the morphological classification. However, placement of several key lineages (genera) confounds morphological classification. In an effort to reconcile morphological classification and molecular systematics, molecular hypotheses are being tested with ever increasing mitochondrial and nuclear datasets. To date, the disparity between morphological classification and molecular systematics remains. This may indicate that this disparity is not due to methodological error or sampling artifacts but may have an evolutionary basis. Molecular systematics has made significant progress in taxonomic coverage of important lineages and the results provides unexpected insights into sub-family and generic relationships. Notably, that many genera or sub-genera are not monophyletic, implying independent evolutionary origins. That traditional sub-family classifications are inadequate as means to understand argasid evolution. As such, the causal factors for the discrepancies observed between morphological and molecular systematics may lie outside conventional taxonomy. The current study unpacks these issues and provide an update on the progress to understand argasid evolution in the light of morphological and molecular systematic disparity.

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

10:20-10:40

Nuttalliellidae in Burmese amber: Implications for tick evolution

Lidia Chitimia-Dobler^{1,2}, Stephan Handschuh³, Jason A. Dunlop⁴, Ronel Pienaar⁵ & Ben J. Mans^{5, 6*}

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Ticks are composed of three extant families (Argasidae, Ixodidae, and Nuttalliellidae) and two extinct families (Deinocrotonidae and Khimairidae). The Nuttalliellidae possess one extant species (*Nuttalliella namaqua*) limited to the Afrotropic region. A basal relationship to the hard and soft tick families and its limited distribution suggested an origin for ticks in the Afrotropics. The Deinocrotonidae has been found in Burmese amber from Myanmar and Iberian amber from Spain, suggesting a wider distribution of the lineage composed of Deinocrotonidae and Nuttalliellidae. The current study describes eight fossils from mid-Cretaceous (ca. 100 Ma) Burmese amber: two *Deinocroton* species, five *Nuttalliella* species, and a new genus and species. The argument is advanced that *Deinocroton* do not warrant its own family, but forms part of the Nuttalliellidae comprising three genera, *Deinocroton*, *Nuttalliella*, and the new genus. Affinities of Burmese tick fossils to the Australasian region, specifically related to rifting of the Burma terrane from northern Australia ~150 million years ago, suggest that *Nuttalliella* had a much wider distribution than its current limited distribution. The distribution of *Nuttalliella* likely stretched from Africa over Antarctica and much of Australia, suggesting that extant members of this family may still be found in Australia. Considerations for the geographic origins of ticks conclude that an Afrotropic origin can as yet not be discarded.

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

10:40-11:00

**Discovery of a novel Mediterranean *Haemaphysalis (Ornithophysalis) doenitzi* group tick species
infesting *Falco eleonora* on Antikythira Island, Greece**

Lidia Chitimia-Dobler^{1,2}, Christos Barboutis³, Anastasios Bounas³, Christina Kassara³, Ben J. Mans^{4,5,6}
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Eleonora's falcon (*Falco eleonora* G  n  , 1839) is a well-known long-distance migrant of the Afro-Palearctic flyway, a summer breeder of the Mediterranean region and North-West Africa and a winter resident of Madagascar and surrounding areas, thus characterized as a double endemic. Within the context of a long-term monitoring and conservation program on Antikythira Island, Greece, which accommodates one of the largest concentrations of breeding pairs of Eleonora's falcons globally, birds were subjected to regular inspections for the presence of ticks from 2017 to 2023. A total of 110 Eleonora's falcons, comprising both adults and subadults (the latter being non-reproductively mature) were captured utilizing mist nets. Of these individuals 25 were tick infested, constituting 22.7% (95% CI: 15.3- 31.7) of the overall sample. In addition, our survey encompassed 11 nestlings, with seven of them exhibiting tick infestations, corresponding to an infestation rate of 63.6% (95% CI: 30.8- 89.1) within this specific cohort. In total, 104 adults and 149 nymphs (all belonging to *Haemaphysalis* genus) were collected. All ticks, apart from two nymphs, exhibited broadly salient palpi and did not possess the pronounced palpal segment 2 spurs or spur-like angles that are characteristic of adults, nymphs, and most larvae of *Rhipistoma*, thus placed them in the *Ornithophysalis* subgenus. Following comprehensive morphological assessment and genetic analysis of the mitochondrial genome by means of next-generation sequencing of both adult and nymphal stages of the ticks, our empirical findings substantiate the delineation of a previously unclassified species, designated as *Haemaphysalis cf. hoodi*. This taxonomic assignment situates the newly described species within the *Ornithophysalis* subgenus and the *Haemaphysalis doenitzi* group, marking its presence for the first time within the Western Palearctic region. It additionally signifies the importance of Falconidae as hosts for tick species within the *H. doenitzi* group.



CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

11:30-11:50

**Fragments of 12s and 16s mitochondrial genes are useless when compared to fragments of
cox 1 in tick taxonomy and diagnosis**

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²Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Hokkaido 060–0818, Japan.

Fragments of 12s RNA and 16s RNA mitochondrial (mt) genes have been used by a tick researchers, sometimes exclusively, for decades. Yet the diagnostic power of 12s and 16s mt genes is incredibly low compared to fragments, even short fragments (say 500 bp), of cytochrome oxidase 1 (*cox 1*). Furthermore, there is a massive data base of *cox 1* sequences for ticks and other creatures in the public domain since *cox1* is the main gene of the Barcode of Life Data System (BOLD) DNA fingerprinting project (<https://www.boldsystems.org>) which has been operating at no charge for two decades now. BOLD contains barcode sequences for well over 300,000 formally described species of animals, plants, fungi, protists, from over 9 million individual specimens. Why tick workers persist with 12s and 16s sequences when the bootstrap support in their phylogenetic trees is so low compared to *cox 1* sequences is a mystery but it seems that some workers have their favourite PCR primers which they do not wish to part with. Yet in our hands *cox 1* sequences are just as easy to amplify by PCR as 12s and 16s sequences; indeed, 12s RNA, 16s RNA and *cox 1* are all genes are from the mitochondrial genome. We will show some of the phylogenetic trees that led us to these strong conclusions. And we will argue that bootstrap support of close to 90% or ideally over 90% is needed to conclude anything scientific from phylogenetic trees. There is no point in sequencing 12s and 16s genes if these sequences provide phylogenetic support of say 60%! Indeed, we note that some journals no longer accept 12s and 16s sequences for papers on the species-level taxonomy of ticks and other Acari. One final plea: please look at sequences previously published and at least make sure your PCR fragment matches that part of the gene that has been sequenced before: *most of us sequence the 5' (front) end of cox 1*.

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

11:50-12:10

***Rhipicephalus sanguineus* s.l transcriptome analysis along with instar from four natural regions of Colombia.**

Gabriel A. Tafur-Gomez^{1,2*}, Marco Antonio Piñeros¹, Yilibeth Franco¹ Camila Domínguez¹, Danny Wilson Sanjuanelo Corredor¹, Carolina Cardona¹, Alejandro Rodriguez³, Alejandro Hoyos-Jaramillo⁴ Rodrigo P. Baptista⁵.

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The tick complex of *Rhipicephalus sanguineus* s.l. comprises species that are widely distributed throughout the world and are important vectors for agents that infect both animals and humans. The 'tropical' lineage in Colombia has demonstrated a significant dispersal capacity across ecological regions of the country, which exhibit notable differences in exposure to environmental pressures. This has resulted in varying densities, community compositions, and reduced genetic diversity, with different patterns of gene expression in response to environmental pressures. These patterns may resemble those observed in continental areas. Consequently, studying the transcriptome of Colombian ticks contributes to understanding gene expression in tick populations exposed to different environmental pressures worldwide. In this study, we collected engorged female ticks from infested dogs in four natural regions of Colombia to establish amplified colonies in rabbits. The instars were collected and conserved at -80°C after challenge. Total RNA was extracted, quantified, and quality verified from these ticks. MACE-seq was used to perform genome-wide gene expression profiling with RNA extracted from tick instars. The libraries were prepared using the Rapid MACE-Seq kit (GenXPro GmbH, Germany) following the manufacturer's protocol for sequencing the 3' end of mRNA transcripts on a NextSeq platform. PCR duplicates were identified using TrueQuant technology and removed from the raw data. The remaining reads were then poly(A)-trimmed, and low-quality reads were discarded. The reads were aligned to the *R. sanguineus* genome (ASM1333969v1), and expression hits were used to predict proteins. The libraries were normalized and compared between instars in each natural region using DEseq2. Transcripts from all ecological regions showed a common expression of upregulated and downregulated transcripts with a significance value of $p < 0.01$, confirmed by RT-PCR. This approach demonstrates variations in transcriptome expression during the development of instar ticks across different ecological regions of Colombia.

Keywords: Colombia, *Rhipicephalus sanguineus*, Brown-Dog-Tick, transcriptome, Tick-Borne Diseases.

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CUBA - 2024

11TH TTP
CONFERENCE

Oral presentations

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

12:10-12:30

**A MALDI-TOF MS approach to differentiate *Amblyomma maculatum* ticks of public health
importance in the United States**

Maria F. B. M. Galletti^{1*}, Andris Atkinson¹, Joy A. Hecht¹, John R. McQuiston², Brendan Headd², Thomas Douglas², Jake Cochran², Bessie H. Blocher¹, Bryan N. Ayres¹, Michelle E. J. Allerdice¹, William L. Nicholson¹, Christopher D. Paddock¹

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During the last several decades, the number of recognized tick-associated pathogens in the United States has risen substantially. In addition, new vector species have been identified, and the geographical ranges of many medically relevant tick species have greatly expanded. Altogether, these events emphasize the need for improved methods to accurately identify tick species of public health importance. Morphological and DNA-based methods currently represent the primary techniques used to identify ticks. However, these methods can be challenging for species separated by extremely subtle morphological characteristics, immature stages, damaged specimens, and tick species for which detailed molecular data are sparse or lacking. Recent morphological and genetic investigations suggest that *Amblyomma maculatum*, the vector of *Rickettsia parkeri* rickettsiosis in the United States, could represent a species complex comprising at least two distinct taxa. In this work, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) was evaluated as a cost-efficient method for the identification and speciation of *A. maculatum* ticks from 13 US states based on the construction of a robust protein reference database. Classical DNA sequencing and pathogen screening work were also evaluated. Their protein reference databases, in addition to the morphological and DNA sequence datasets were deposited into the CDC MicrobeNet, a publicly accessible free on-line resource that incorporates collective data from thousands of bacterial and fungal organisms that allows public health and scientific communities to perform microorganism identification based on a searchable MALDI-TOF protein reference database. The tick module of MicrobeNet containing protein-based reference databases of four species of public health importance in addition to curated pathogen-related information will be presented. This is the first integration of information for medically relevant arthropods into an internationally accessed platform. Efforts are ongoing to collect and incorporate data for additional tick species in the US and abroad to strengthen vector identification and facilitate tick surveillance programs.

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

12:30-12:50

Integrated approaches for surveillance of ticks and tick-borne diseases (Arachnida: Ixodidae) of livestock importance

Luis M. Hernández-Triana ^{(1)*}, Ter Apaa ⁽¹⁾, Ben Jones ⁽¹⁾, L.P. Phipps ⁽¹⁾, Kayleigh Hansford ⁽²⁾, Jolyon Medlock ⁽²⁾, Alex G. C. Vaux ⁽²⁾, Sanam Sewgobind ⁽¹⁾, Ana M. Palomar ⁽³⁾, José A. Oteo ⁽³⁾, Insiyah Parekh ⁽¹⁾, Karen L. Mansfield ⁽¹⁾, Nicholas Johnson ⁽¹⁾

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Abstract

Ticks are of medical and veterinary concern due to their role as vectors of a wide range of pathogens. Therefore, accurate identification of tick species is essential for surveillance and disease control programs. In this study, an integrated approach was implemented for the identification of ticks associated with tick-borne diseases of livestock and wildlife. Morphological traits in combination with genetic variability within the cytochrome c oxidase I gene were employed to develop a DNA barcoding library for the identification of exotic and UK. Molecular analyses were undertaken to detect specific pathogens either in ticks, or tissues and/or blood samples from infected animals, together with bioinformatic analysis. In total, 30 tick species were analyzed for the barcoding gene profile in nine genera. Phylogenetic analysis confirmed discrete clustering of sequences according to species, except for *Rhipicephalus sanguineus* s.l., *Ixodes vespertilionis* and *Ixodes* nr. *affinis*. Analysis of *I. ricinus* from National Parks and Areas of Outstanding beauty in Wales and England revealed the presence, of *Babesia capreoli*, *B. divergens*, *B. venatorum* and *B. odocoilei*. Illumina sequencing and bioinformatic analysis of *I. ricinus* from UK revealed the presence of Phlebovirus and Norway virus. Examination of fox carcasses (n=13) showed they were infested with *I. canisuga*, *I. hexagonus* and *I. ricinus*, of which nine carcasses (69.2%) were infected with *B. vulpes*. Analysis of blood and tissue taken from livestock with febrile disease (n=22) confirmed *B. divergens* in four cattle samples (18%), and *Anaplasma phagocytophilum* was detected in one sheep blood (4.5%). Testing of RNA extracted from livestock identified one sheep infected with Louping Ill virus; the same sample was infected with *A. phagocytophilum*. In conclusion, use of morphology, COI DNA barcoding, and targeted molecular assays was an effective approach for distinguishing ticks, cryptic diversity, and the detection of tick-borne pathogens in ticks and animal species.



CU3A - 2024

11TH TTP
CONFERENCE

Oral presentations

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

12:50-13:10

**A core genome MLST scheme provides higher resolution insights into the *Borrelia burgdorferi*
sensu lato species complex**

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Abstract

The causative agent of Lyme borreliosis are bacteria belonging to the *Borrelia burgdorferi* s.l. species complex. Molecular typing is key for bacterial characterization and a multilocus sequence typing (MLST) scheme based on eight chromosomal housekeeping genes, established by Margos et al. in 2008, is the gold standard for *Borrelia*. Since 2015 the PubMLST website has hosted the *Borrelia* MLST data. However, whole genome sequencing provides typing schemes with higher resolution than the typical MLST schemes. We developed a 639-gene core genome MLST (cgMLST) scheme which extends the advantages of MLST to a genomic scale.

Good quality genomes currently available in the *Borrelia* PubMLST database (n=174, 2023-12-28) served as primary genome set containing 17 species of the *B. burgdorferi* s.l. complex. All chromosomal coding sequences (CDS) of *B. burgdorferi* s.s. B31 from Genbank (n=815) were used as the primary loci set.

We identified 639 loci present in at least 95% of the primary genome set (n=174) that were included in the cgMLST scheme. As the primary validation, phylogenetic analyses were conducted, showing that samples clustered according to species and the division into two major clades (North American and European). These findings were in accordance with the results based on the 8-loci MLST, but the 639-gene cgMLST led to a higher resolution. Furthermore, we showed that recombination events do not affect the phylogeny based on the cgMLST scheme.

The developed 639-loci cgMLST scheme enables unambiguous genotyping and fine-scale phylogenetic analyses at a high resolution. This new scheme can be applied to whole genome data and is an addition to the 8-loci MLST scheme that is suited for environmental samples (tick, host, human) without prior culturing. Both schemes are publicly available through the *Borrelia* PubMLST website.

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

13:10-13:30

***Borrelia tillae* – revival of a *Borrelia* species from South Africa**

Volker Fingerle¹, Sabrina Hepner¹, Sylvia Stockmeier¹, Cecilia Hizo-Teufel¹, Christine Hartberger¹, Peter Kraiczy², Felicia Schmidt², Tom Schwan³, Ben Mans⁴, Ronel Pienaar⁴, Gabriele Margos¹

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Abstract

Ornithodoros zumpti Heisch and Guggisberg 1953 is a soft tick species that has been found in nests of rodents in Eastern South Africa. In 1961 Zumpt and Organ described the transmission of a relapsing fever *Borrelia* species from *O. zumpti* into mice. The *Borrelia* species showed differences in a complement-fixation test compared to *Borrelia duttonii* and was named *Borrelia tillae*. It was infectious in laboratory mice and multimammate rats. An attempt to infect *O. moubata* proved unsuccessful but it was successfully isolated from *O. zumpti* (1).

At the NRC Germany, *Borrelia* bacteria labelled *B. tillae* were cultured. We sequenced PCR products of 16S rRNA, *flaB* and multilocus sequence typing (MLST) loci and generated phylogenetic trees. An *O. zumpti* colony was screened by PCR targeting and sequencing the *Borrelia* 16S rRNA locus. Furthermore, *Borrelia tillae* was also exposed *in vitro* to complement-active human serum.

No close matches to 16S rRNA, *flaB* or MLST loci were found in BLAST searches. In phylogenies of those sequences, *B. tillae* sequences cluster on their own branches. Borrelial 16S rRNA sequences from an *O. zumpti* colony were identical to the sequence obtained from the cultured isolate.

Sequence comparison provides evidence that the recovered isolate differs from all *Borrelia* spp. for which sequence data exist. Identical 16S rRNA sequences from an *O. zumpti* colony to the cultured isolate strongly support that we recovered *B. tillae*. To consolidate our findings with previous descriptions, we endeavour to isolate viable spirochetes from *O. zumpti*. By using serum bactericidal assays, *B. tillae* strongly resists complement-mediated killing by human serum but no data are currently available regarding human pathogenicity. Detailed bioinformatics are ongoing to finalized the genetic data obtained and to characterize *B. tillae* in more detail.

(1) Reference: Zumpt and Organ, 1961, S Afr J Lab Clin Med 7, 31-35

Symposium Tick-borne diseases of bovines
Room Internacional II, Meliá Internacional Varadero

9:30-10:00

Tick borne pathogens in ruminants horses and ticks in Croatia

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Anaplasmosis, babesiosis, and theileriosis are (re) emerging diseases affecting ruminants and horses in Europe, which are associated with climate change and changes in animal husbandry practices. Recent reports have confirmed cases of bovine mortality caused by *Anaplasma marginale*, *Anaplasma bovis*, and *Theileria orientalis* (buffeli/sergenti). Additionally, sheep infections caused by *Anaplasma ovis* have also been recognized. Between 2022 and 2024., 8.5% (4 out of 47) of examined cattle had either a single infection or co-infection with *A. marginale*,

T. orientalis, *Babesia divergens*, and *Mycoplasma bovis*. These infections were associated with post-mortem signs consistent with hemolytic anaemia. Various genotypes of *Theileria equi* and *Babesia caballi* were detected in both diseased and healthy horses. The present study aimed to examine the occurrence of TBDs of horses and ruminants in tick obtained from the environment, as well as from other domestic and wild animals across Croatia. Following morphological identification, each tick was confirmed through sequencing a portion of the COI and 16SrRNA. A total of 657 ticks were identified, consisting of 72 *Rhipicephalus sanguineus*, 150 *Rh. turanicus*, 69 *Rh. bursa*, 2 *Haemaphysalis concinna*, 6 *Hae. inermis*, 8 *Hae parva*, 35 *Hy. narginatum*, 4 *Ixodes gibbosus*, one *I. rugicolis*, 6 *I. ventalloi*, 23 *I. canisuga*, 19 *I. hexagonus*, 106 *I. ricinus*, 15 *Dermacentor marginatus*, and 141 *D. reticulatus*. Through molecular analysis of each tick, we identified the presence of *Theileria equi*, *T. ovis*, *T. orientalis*, *T. capreoli*, *Babesia cf. crassa*, *B. ovis*, and *B. venatorum*. Additionally, we detected the presence of *Anaplasma phagocytophilum*, *Anaplasma ovis*, *Ehrlichia canis*, and *Ehrlichia* sp. By current research, we have successfully identified multiple pathogens present in ticks that could potentially threaten the health of ruminants and horses. However, we have not detected the presence of *A. marginale* and *B. caballi*. The *E. canis* was discovered in Croatia for the first time in *Rh. bursa*, collected from sheep and chamois.

Other causal agents such as *Anaplasma phagocytophilum*, *A. centrale*, *B. venatorum*, and *Babesia* cf. *crassa* *Babesia ovis*, *Babesia divergens*, and *Theileria ovis* were all detected in ruminants and ticks in Croatia, demonstrating the wide range of infections transmitted by ticks that can cause disease and death in ruminants. We will discuss current knowledge concerning the occurrence and relevance of tick-borne ruminant pathogens in this review paper.

Symposium Tick-borne diseases of bovines
Room Internacional II, Meliá Internacional Varadero

10:00-10:30

**The roles of vitellogenins and their related molecules on *Babesia* transmission in
Haemaphysalis longicornis ticks**

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Transovarial transmission of *Babesia* parasites has been experimentally demonstrated using larvae obtained from *Haemaphysalis longicornis* female ticks parasitized on cattle infected with *Babesia ovata*. However, the molecular mechanisms underlying the transovarial transmission remain largely unclear. We previously showed that vitellogenin (Vg) and its receptor, which are essential for oogenesis, are key factors involved in *Babesia* infection in the ovary of *H. longicornis*. For instance, Vg uptake from the hemolymph to the ovary was suppressed in the presence of *B. ovata*, indicating that accumulated Vg protein is associated with *Babesia* infection or transmission in the tick body. Three Vg genes (*HIVg-1*, *HIVg-2*, and *HIVg-3*) have been identified from *H. longicornis* so far, however, the individual roles of Vgs in *Babesia*-infected ticks are still unknown. In the present study, we focus on midgut-specific Vg of *H. longicornis* (*HIVg-1*) to understand the roles of oogenesis-associated molecules in *Babesia*-infected ticks. *Haemaphysalis longicornis* (parthenogenetic strain) infected with *B. ovata* (*in vitro* cultured Miyake strain) was prepared by a semi-artificial feeding system and the *HIVg-1* mRNA expression in the midgut of *B. ovata*-infected ticks was examined. *HIVg-1* gene expression levels in the *B. ovata*-infected ticks were significantly upregulated compared with *B. ovata*-free ticks at 1 and 2 days after engorgement. Subsequently, *HIVg-1* gene knockdown mediated by RNA interference (RNAi) was performed to predict the function of *HIVg-1* in *B. ovata*-infected ticks. Interestingly, relative detection levels of *B. ovata* DNA in *HIVg-1* RNAi ticks appeared to be higher compared with *B. ovata*-free ticks at 1 and 2 days after engorgement. These results indicate that *HIVg-1* might regulate tissue-to-tissue migration of *B. ovata* in the tick body. Our data propose the hypothesis that each organ-specific Vg has individual roles during *Babesia* infection or transmission.

Symposium Tick-borne diseases of bovines
Room Internacional II, Meliá Internacional Varadero

10:30-11:00

Abundance and distribution of *Rhipicephalus microplus* complex infesting *Bos indicus* animals from 10 cattle farms in Cambodia

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Cattle tick infestations pose a significant threat to livestock health and productivity worldwide. These parasitic arthropods, primarily belonging to the *Rhipicephalus microplus* complex, feed on blood and can transmit various diseases to cattle, such as anaplasmosis, babesiosis, ehrlichiosis, and theileriosis. The economic impact of cattle tick infestation can reduce meat and milk production, increase veterinary costs, and potential trade restrictions. The objective of this study was to describe the abundance and distribution of cattle tick species on bovine animals in Cambodia.

A cross-sectional study was conducted during the dry and rainy season from November 2022 to September 2023. Ticks were collected from 10 animals in 10 provinces, except Svay Rieng (n=5), and preserved in 75% ethanol for morphological identification. A total of 6,458 ticks (4,248 adults, 2,183 nymphs, 27 larvae) were collected from 190 animals. Three tick species were identified to the species level: *Rhipicephalus microplus* (n= 2,758; 42.71%), *Rhipicephalus australis* (n=1373; 21.26%), and *Rhipicephalus haemaphysaloides* (n=13, 0.2%). In addition to the three species, some individuals, mainly nymphs and larvae were identified as *Rhipicephalus* sp. (n=2,314; 35.83%). Further statistical analysis is currently processed to determine the impact of meteorological factors, specific species distribution on the hosts in each province and will be presented during the conference. This is a very first study highlighting the absence of knowledge, and further field study must be done, including pathogen determination, in order to better characterize the risk.



CUBA - 2024

Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

11:30-11:50

Internal organ metagenomics of adult semi engorged *Rhipicephalus australis* female ticks.

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Cattle ticks and tick-borne diseases are a global challenge. *Rhipicephalus australis* (Australian cattle tick) was introduced into Australia and belongs to a broader cryptic species of *R. microplus* with global distribution causing USD\$22–30b per annum in losses. The tick microbiome is a target for potential control strategies, but few studies have investigated the *R. microplus* species complex and no studies have utilised un-biased metagenome approaches. Gut and salivary gland tissues were dissected from 60 semi engorged adult female ticks, with 3 replicates for each tissue. Extracted DNA was sequenced using long read Oxford Nanopore platforms with adaptive sampling to deplete host (tick) DNA, utilising a draft genome of *R. australis*. No statistical difference in alpha diversity between gut and SG samples ($p = 0.12$) was observed, however differences between genera and species were evident. The dominant genus in salivary glands is *Coxiella* spp. (mean abundance, 87%), contrasting with the gut samples where *Staphylococcus* spp. (mean abundance, 49%) and *Escherichia* spp. (mean abundance, 31%) dominate. *Staphylococcus* spp., *Xylosus* spp., *S. chromogenes* and *E. coli* were prevalent in gut, while *C. mudrowiae* was the most prevalent *Coxiella* spp. in the salivary glands. Potential pathogenic species included *Corynebacterium resistens* and *Borrelia* spp. Analysis of the gut and SG tissues has revealed genera and species present in each tissue, most interestingly the dominance of the endosymbiotic *Coxiella* spp. in the salivary glands. The finding of *Coxiella* species concurs with microbiome research findings and these species have been implicated in Vitamin B synthesis including the cofactors biotin and riboflavin required for tick survival. Future research will determine whether the endosymbiotic species identified here are similar to those found in other tick species or whether they are species specific, and the data will be mined for the presence of tick Vitamin B genes.



CUBA - 2024

Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

11:50-12:10

RNA Virome of *Ixodes ricinus* in Slovenia

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Tick-borne viruses are of particular public health concern as many of them are agents of emerging and re-emerging infectious diseases. Recent studies suggest that *I. ricinus* ticks in Europe harbour undiscovered viruses. Slovenia has been recognised, as an endemic area for tick-borne encephalitis (TBE) for more than 70 years, and the incidence of TBE is one of the highest in Europe. It is also highly endemic for Lyme borreliosis and anaplasmosis, and rarely *Babesia* sp. parasites and *Francisella tularensis* are also detected. In this study, we aimed to analyse the virome of *I. ricinus* ticks sampled in Slovenia. We were interested in the composition of the RNA virome, the comparison between different developmental stages of ticks (larvae, nymphs and adults) and geographical locations. Virus families *Nairoviridae*, *Phenuiviridae* and *Partitiviridae* were most abundant in *I. ricinus* ticks. We analysed RdRP sequences belonging to the families *Nairoviridae* and *Phenuiviridae* families. The diversity of nairoviruses was relatively low and showed similarity to the sequences from of the genus *Norwavirus* detected in ticks from central and northern Europe and Russia. The majority of the viruses from the family *Phenuiviridae* detected in Slovenian ticks showed the highest similarity to the genus *Ixovirus*. In addition, Uukuniemi virus was detected in *I. ricinus* at a single sampling site. Tick-borne encephalitis virus was the only flavivirus detected. Viruses of the order *Reovirales* were also detected, including those belonging of the genus *Coltivirus*. Contigs similar to virus sequences of the taxa *Rhabdoviridae*, unclassified *Bunyavirales* and *Iflavirus* were also confirmed in Slovenian ticks. Further analysis is needed to understand the natural circulation of these viruses and their potential pathogenicity.

Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

12:10-12:30

A Multiplex Amplicon Nanopore Sequencing Assay for Characterisation of UK Tick Species, Their Microbiomes, and Bloodmeal Origin.

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In the UK, tick surveillance is routinely limited to morphological identification and targeted qPCR assays. Although these methods give reliable data on pathogen prevalence and presence in tick populations, the number of targets that can be tested using this approach is limited. A multiplex PCR based sequencing panel using Nanopore was developed to be used in routine surveillance in the UK, to assess public health risk by monitoring tick species and pathogens present, as well as their source in nature by revealing last host.

This sequencing panel was used to characterise ticks collected from both game animals and other wildlife found in the UK. Little is known about the role of game animals and birds in tick pathogen cycles, despite their close contact with humans. *Borrelia afzelii*, *B. valaisiana* and *B. garinii* are known to be associated with birds and have previously been detected in pheasants in the UK. However, it is currently unknown whether there are any other species or potentially pathogenic bacteria present in game birds. Ticks at both the larval and nymphal stage feed on ground-nesting birds, so it can be assumed that there is some contribution to pathogen prevalence. Deer have been widely studied in their role as dilution hosts for *Borrelia* and are competent hosts of *Anaplasma phagocytophilum* in the UK. Findings from this study show that *Ixodes ricinus* is the most abundant tick species feeding on animal and bird hosts, but *I. frontalis* and *I. hexagonus* were also found. Source of last host was confirmed in 88% of ticks, as well as identifying other possible hosts from previous developmental stages. Microbiome of ticks did not differ significantly across animal hosts but showed similarities within animal hosts and revealed pathogenic genera, with some ticks showing evidence of possible co-infections.

Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

12:30-12:50

Metagenomic Analysis of *Rickettsia* Plasmid Sequences Obtained Directly from Ticks

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The advent of Next Generation Sequencing (NGS) coupled with advanced bioinformatic tools has facilitated the acquisition of sequences from microorganisms in both human and vector samples without their prior cultivation or isolation (metagenomic assembled genome-MAG). We evaluated the accuracy of this approach by comparison of MAG sequences derived from single *Amblyomma americanum*, *A. maculatum*, *D. occidentalis*, and *D. variabilis* adults. Sequences (2.9-6.2 million, 80-272 median bp) were obtained with low coverage chips on the Ion Torrent PGM platform, in some cases following Repli-G DNA amplification to obtain enough tick DNA for PCR amplified library preparation. All three *R. amblyommatis* plasmids (18, 23, 32 kb) were recovered with few differences from *A. americanum* *R. amblyommatis* GAT-3OV isolate. *R. rhipicephali* from *D. occidentalis* (California) was 32 bp smaller than the 15 Kb plasmid from *R. rhipicephali* 3-7-F6 CWPP isolated from a *Rhipicephalus sanguineus* tick (Mississippi) but they differed in annotation. The *D. variabilis* plasmid differed only in a few SNPs and polynucleotide runs from the *D. variabilis* *R. bellii* OSU 85-389 isolate plasmid (48.9 Kb) obtained 70 miles and 60 years apart in Ohio. *A. maculatum* (Oklahoma) was found to have chromosome sequences expected from conserved *R. andeanae* sequences. It also had plasmid sequences most closely related to those found in *R. amblyommatis*, *R. massiliae*, *R. rhipicephali*, and *R. aeschlimanii* plasmids. Despite bioinformatic efforts to assemble it and to link contigs by long-range PCR, we could not determine whether it was one large plasmid or three. The new *R. andeanae* chromosome and plasmid sequences will be useful in determining whether this species is variable in the many tick species (*Amblyomma* sp., *Rhipicephalus*, *Ixodes*) in which it has been detected. Determining MAG sequences should become a routine approach for detecting new rickettsial plasmids and for population genetic studies of *Rickettsia*.

Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

12:50-13:10

Bacterial microbiota of ticks infesting humans in the Yozgat province of Türkiye

Djursun KARASARTOVA^{1*}, Gonul ASLAN-AKVERAN^{2*}, Hulya SIMSEK³, Sabiha SENSOZ⁴, Adem KESKIN⁵, Levent ALBAYRAK⁶, Emre GOKCEN⁶, Ibrahim CALTEKIN⁶, Kosta Y MUMCUOGLU⁷, Aysegül TAYLAN-OZKAN⁸

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Background: Analyzing the microbiota associated with ticks and comprehending the intricate interplay between ticks and their bacterial endosymbionts are essential for host survival, development, and the transmission of pathogens. In the present study, the bacterial microbiota of ticks infesting humans in the Yozgat Province of Türkiye was analyzed.

Methods: Ticks were collected from 54 patients who presented at the Emergency Service of the Yozgat Bozok University Research and Training Hospital between March and November 2019. Using the 16S rRNA V3-V4 region amplification in a MiSeq Illumina, tick pools consisting of 1-28 specimens were analyzed.

Results: The operational taxonomic units were classified into 20 phyla, 41 classes, 94 orders, 168 families, 344 genera, and 452 species. Notably, the most abundant phyla were Proteobacteria, Actinobacteria, Bacteroidota, and Firmicutes, while the most abundant species were *Francisella* sp. and *Candidatus* Midichloria mitochondrii. The highest microbial diversity was found in *Hyalomma marginatum* as evidenced by a higher number of both operational taxonomic units and measures of species richness.

Conclusions: The findings will aid in developing effective surveillance and control measures to mitigate tick-borne diseases, including rickettsiosis, Lyme disease, and Crimean-Congo hemorrhagic fever, especially in a hyperendemic region like the Yozgat Province.

Key words: Microbiota, ticks, humans, tick-borne diseases

Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

13:10-13:30

Microbiome of the bush tick (*Haemaphysalis longicornis*): the current state of play revisited

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Haemaphysalis longicornis, a three-host tick, is an important vector of numerous bacterial, protozoal, and viral pathogens, and widely distributed in Australia, eastern Asia, New Zealand, and the USA. We systematically reviewed the literature on the microbiome of *H. longicornis* using PRISMA guidelines. Our inclusion criteria identified 240 studies from four databases (Web of Science, PubMed, Scopus and CAB Direct). Most studies focused on identifying "pathogen" components of the tick microbiome, including bacteria (*Anaplasma*, *Borrelia*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Francisella* and *Rickettsia*), viruses (Dabie bandavirus, Heartland bandavirus, Powassan virus and Nairobi sheep disease virus) and protists (*Babesia*, *Hepatozoon*, *Theileria* and *Toxoplasma*). Additionally, endosymbionts such as *Arsenophonus*-like, *Coxiella*-like and *Rickettsia*-like were also detected in ticks. Our findings suggest that the microbiome of *H. longicornis* plays a significant role in tick's life cycle and its capability to transmit several microorganisms to humans and animals. Future investigations should encompass the "non-pathogen" microbiome components of *H. longicornis* to understand their role in tick biology and the transmission of pathogens to develop sustainable strategies for controlling ticks and tick-borne diseases.



CUBA - 2024

Symposium Tick microbiota

Monday September 2nd, Room Cetro - Meliá Cohiba

P01

Trying to disentangle the transmission cycle of *Anaplasma marginale* through *Rhipicephalus microplus* microbiome characterization at the organ scale

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Rhipicephalus microplus, a one-host tick from the *Ixodidae* family, is the most important vector for *Anaplasma marginale* in cattle. The study of tick microbiome is an emergent approach for understanding vector-pathogen interaction and defining new targets for control strategies. We aimed to study the influence of *A. marginale* infection over *R. microplus* bacterial microbiome at the organ scale.

Engorged female ticks were collected from randomly selected bovine hosts in an enzootic region from Argentina. The arthropod surface was disinfected previous to dissection of ovaries (Ov) and salivary glands (SG). Samples were classified as: infected (iOv and iSG) or uninfected (uOv and uSG) after *A. marginale* detection using *msp1β* PCR. We systematically collected control samples along the whole protocol to identify contamination. The bacterial community composition was explored by Illumina metabarcoding, targeting the *16SrRNA* V3-V4 region. We removed likely contamination with 'decontam' package implemented in Rstudio environment and performed microbiome analysis using QIIME2.0. While alpha diversity in SG was higher than in Ov, no statistical differences were found between uninfected (uOv and uSG) and infected (iOv and iSG) conditions. On the contrary, beta diversity displayed significant differences between uninfected and infected conditions in both tissues. In SG we found 63 shared taxa (92.64%), 1 (1.5%) taxon exclusive to uSG and 4 (5.86%) taxa exclusive to iSG. In Ov we found 48 taxa shared (44%), 39 (36%) taxon exclusive to uOv and 22 (20%) taxa exclusive to iOv. Co-occurrence networks were developed to examine microbial community interactions. Interestingly, *A. marginale* infection in SG induces an increase in network complexity, whereas in Ov, *A. marginale* infection leads to a reduction. *Coxiella* endosymbiont appears in SG and Ov samples under both conditions. These preliminary data sets represent convincing evidence of *R. microplus* microbiome modulation by *A. marginale* at the organ level.



CUBA - 2024

Symposium Tick microbiota

Monday September 2nd, Room Cetro - Meliá Cohiba

P02

Unraveling holobiont-holobiont interactions in host-ectoparasite systems

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The holobiont theory suggests that an animal and its associated microorganisms — such as bacteria, viruses, fungi, and other microscopic life forms — together constitute a single ecological entity, mutually influencing each other's health, growth, and evolution. Our research explored this theory within host-parasite relationships, proposing that the microbial components of one holobiont could significantly impact, and be influenced by, another. To test this, we examined the interactions between two ectoparasites, the ticks *Ixodes ricinus* and the mites *Varroa destructor*, with their hosts, the mouse (*Mus musculus*) and the honeybee (*Apis mellifera*), respectively. Using next-generation sequencing to analyze the bacterial 16S rRNA from mouse feces and ticks, as well as from *Varroa* mites and honeybees, we were able to characterize the microbial landscape of each holobiont. Our analysis revealed not only niche differentiation but also shared microbial interaction patterns between hosts and their ectoparasites. Moreover, altering the mouse gut microbiota through oral administration of new microbes influenced the taxonomic contributions to central metabolic pathways in both the mouse and the tick, demonstrating a cascading effect on the ectoparasite's microbiome. We also investigated how pathogen infections, known to modify host-microbiome interactions, affect these dynamics, using *Paenibacillus* in bee-*Varroa* system and *Borrelia* in mouse-tick system. Infections altered the microbial similarity patterns shared between host and ectoparasite, and alterations in the mouse gut microbiota modified the tick microbiota and reduced *Borrelia* infection in the vector. This research adds a significant dimension to holobiont theory by showing that the microbial interactions within host-parasite systems are not isolated. Instead, they are interconnected, with changes in one holobiont's microbiome having the potential to influence another's. This finding underscores the complexity of ecological and evolutionary interactions in nature, emphasizing the holistic approach needed to understand and potentially manipulate these relationships for health and disease management.

Research funding: APVV-19-0440, APVV-17-0372.

Symposium Tick microbiota

Monday September 2nd, Room Cetro - Meliá Cohiba

P03

Culture-dependent analysis of the microbiome of *Amblyomma aureolatum* and *Amblyomma sculptum*

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Rickettsia rickettsii, the causative agent of Rocky Mountain spotted fever, is transmitted by the ticks *Amblyomma sculptum* and *Amblyomma aureolatum* in Brazil. *A. aureolatum* is highly susceptible to *R. rickettsii*, while *A. sculptum* is partially refractory to this pathogen. It has been hypothesized that differences in the susceptibility to these bacteria between the two tick species are at least in part due to the different microbiome composition in the tick midgut.

In this study, the microbiome of *A. aureolatum* and *A. sculptum* (nymphs, males, and females) from a laboratory colony was analyzed by a culture-dependent approach. Ticks (n=20 for each species and developmental stage) were surface sterilized, homogenized, and spread plated on Trypticase soy agar, Bile esculine agar, and McConkey agar. Colony forming units were counted, morphologically different colonies were isolated and identified by MALDI-TOFF and PCR amplification and sequencing of 16S rDNA with the universal primers.

Our results show that the microbiota of these two species is very different with *A. aureolatum* having dominant Gram-negative bacterial taxa *Pseudomonas* sp. and *Stenotrophomonas* while the microbiota of *A. sculptum* was represented mainly by Gram-positive bacteria such as *Mammaliococcus* sp., *Brevibacterium* sp., and *Staphylococcus* sp.

Our data show that the culturable microbiota of *A. aureolatum* and *A. sculptum* is vastly different and this may indeed influence their susceptibility to *R. rickettsii*. Our follow up study is focused on the tick microbiome analysis by the culture-independent approach and this will also include ticks collected from the field.

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Symposium Tick Physiology and Metabolism
Room Cetro, Meliá Cohiba

P04

Kinin neuro peptide in the midgut endocrine cells and innervation of salivary gland of *Ixodes ricinus*

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Abstract

Neuropeptides and their receptor are the key regulators of arthropod physiology coordinating important biological processes including reproduction, development, molting, behavior, and feeding. Invertebrate kinin neuro peptides are well known for their myotropic activities and maintain diverse physiological roles including hindgut contractions, diuresis, feeding, ecdysis and digestive enzyme release. Kinin and its receptor were identified in various tick species, however, their physiological functions are not well defined. In this study, we molecularly identified transcript sequence encoding *Ixodes ricinus* kinin peptides and, using *in situ* hybridization, we visualized the kinin mRNA in specific synganglion neurons. Interestingly, the presence of kinin mRNA in axonal projection within the synganglion lobes suggests active axonal mRNA transport. The anti-kinin antibody not only validated the *in situ* hybridization findings in the neuronal bodies but also revealed rich axonal arborization on the dorso-lateral surface of the synganglion. Subsequently, using immunogold transmission electron microscopy we revealed that these axons terminate on dorsal perineurium facing the acellular neural lamella. In addition, the anti-kinin antibody also identified distinct endocrine cells within the midgut. Moreover, it delineated axonal processes exclusively extending to type II salivary gland acini in *I. ricinus* female. Through phylogenetic analysis, we identified a putative *I. ricinus* kinin receptor and tested its activities in a heterologous expression system using an aequorin reporter. Furthermore, qRT-PCR was employed to investigate the tissue-specific expression of kinin receptor. Our results shed light on the different roles of kinin in tick biology advancing our knowledge of this neuropeptide family in arthropods.

Symposium Tick Physiology and Metabolism
Room Cetro, Meliá Cohiba

P05

Novel neuropeptides involved in the salivary gland innervation of soft tick *Ornithodoros moubata*

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Neuropeptides are the most important signaling molecules regulating key physiological functions in invertebrates. Although the research of neuropeptides in hard ticks has significantly advanced in the last two decades, this scientific field in soft ticks largely remains neglected. In this study we investigated neuropeptidergic axonal connections between the synganglion and salivary gland in nymphal stages of African tick species *Ornithodoros moubata* (Murray, 1877). In total, five different neuropeptide immunoreactivities were found in innervations of type II acini in *O. moubata* salivary glands. Specifically, colocalizations of SIFamide with myoinhibitory peptide (MIP) in axons originating from protocerebral neurons were identified. Another distinct immunoreactive projections were recognized by antibodies against FGLamide-related allatostatin (FGLa/AST), diuretic hormone 31 and EFLamide, however the origin of these axons was not identified. In addition, distinct solo EFLamide-immunoreactive projections were also spotted in type II acini. Immunoreactive axons for SIFamide and FGLa/AST in the acini were confirmed by immunogold transmission electron microscopy. Lastly, anti-tachykinin antibody recognized robust axon running across the salivary glands however, did not terminate within the individual salivary gland acini. Mining of neuropeptide sequences in *O. moubata* genome, transcriptomes of 18 soft ticks as well as *Nuttalliella namaqua* revealed high conservation of these salivary-gland innervating neuropeptides indicating their common functions. Our study has revealed that soft ticks utilize different sets of neuropeptides for their salivary gland control than hard ticks, likely reflecting their distinct feeding strategy. We propose that a deeper understanding of the mode of action of neuropeptides in the control of salivary glands may lead to the development of better control measures of these medically important arthropods. Research funded by ANR-10-LABX-62-IBEID; ANR-21-CE14-0012; APVV-17-0372; APVV-19-0440; VVGS-2023-2703 and VZDstipVS022.

Symposium Tick Physiology and Metabolism
Room Cetro, Meliá Cohiba

P06

Thyropin and cystatin proteins from *Ixodes ricinus* saliva: highly selective inhibition of host cathepsins explained by 3D structures

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Protease inhibitors are important component proteins of tick saliva. Here we present two novel members of the cystatin and thyropin families of protease inhibitors from the tick *Ixodes ricinus*. Ricistatin from the cystatin family has potent immunosuppressive and anti-inflammatory activity, while the physiological role of the thyropin IrThy remains to be elucidated. Recombinant Ricistatin and IrThy were found to inhibit human host-derived cysteine proteases with high specificity for cathepsins possessing endopeptidase activity. This inhibitory specificity is clearly distinct from a broader specificity pattern of cystatin and thyropin homologs expressed by the host. We determined the spatial structures of protease-bound Ricistatin and IrThy by protein crystallography and solution NMR spectroscopy. This allowed us to describe the three-loop reactive centers on the inhibitors and explain their binding specificity. The work provides the first structural insights into the unique selectivity of tick salivary inhibitors targeting cysteine cathepsins and suggests their convergent functional evolution.

Symposium Tick Physiology and Metabolism
Room Cetro, Meliá Cohiba

P07

Differential behavioral responses of ticks to radiofrequency electromagnetic radiation exposure

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Artificial radiofrequency electromagnetic radiation (RF-EMR) is a relatively new environmental factor affecting living organisms. Its effects have been the subject of research conducted on a wide range of animals, including vertebrates and invertebrates. Behavioral experiments on ticks indicate their affinity to radiation. Our study builds upon this and aims to investigate the influence of RF-EMR on the behavior of four sympatric tick species at interspecific and gender levels. Additionally, we aimed to expand knowledge of tick reactions by recording to our best knowledge the first behavior examination of *Dermacentor marginatus* and *Haemaphysalis inermis* species under radiation exposure. In total, we tested 1200 ticks belonging to four species: *Ixodes ricinus*, *Dermacentor reticulatus*, *D. marginatus*, and *H. inermis*. 300 adult individuals of each species were tested using apparatus allowing ticks to choose between an exposed area or an area shaded by a 1mm layer of copper. Ticks were exposed to electromagnetic radiation at a frequency of 900 MHz for 24 hours. We recorded the position of individuals within modules and statistically evaluated the data using the Chi-square test. A significant preference for the exposed area was observed in both male and female *I. ricinus* ticks (males: $P=0.022$; females: $P=0.001$). Male *D. reticulatus* ($P<0.000$) and *D. marginatus* ($P=0.050$) also exhibited an affinity for radiation. Conversely, we did not observe a statistically significant response to radiation exposure in female *D. reticulatus* ($P=1$), *D. marginatus* ($P=0.253$), or in both genders of *H. inermis* (males: $P=0.870$; females: $P=0.870$). The results of the study support the hypothesis that ticks perceive electromagnetic fields, and the observed differences in reaction among species and genders have the potential to help us understand the mechanism of electroreception by comparing species with strong and weak reactions to RF-EMR. The implementation of this study was supported by grants APVV-17-0372, APVV-19-0440, and VVGS-PF-2022-2135.

Symposium Tick Physiology and Metabolism
Room Cetro, Meliá Cohiba

P08

Dynamic ultrastructural changes in the midgut of *Ixodes ricinus* nymphs at different feeding stages

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Ticks are ectoparasites that depend on the blood of their host, from which they obtain essential nutrients for their life cycle. The tick gut undergoes morphological changes during feeding to ensure the gradual digestion of the blood meal, the long-term storage of undigested blood (lumen) and a food reserve in the form of lipid vesicles. Ticks are also dependent on the host's hemoglobin, as they have lost the ability to synthesize their own heme over the course of evolution. Our understanding of lipid metabolism during nymphal feeding and metamorphosis and the necessity of heme for development of nymphs remains unclear. In this study, we used light and transmission electron microscopy (TEM) to investigate the morphological changes in the nymphal tick gut from the unfed stage to four weeks post-detachment. We investigated the dynamics of lipid droplets (LD) that play a central role in the management of lipid reserves, and the distribution of hemoglobin during the different feeding stages of ticks by TEM immunolabeling on the Tokuyasu cryosections. In parallel, we performed serial block-face scanning electron microscopy followed by 3D image reconstruction and TEM tomography of the nymphs' gut (48 hours after feeding).

The results of our work presented here show the distribution of hemoglobin, albumin and lipid droplets within the midgut cells before, during and after feeding. All these components of the midgut play a crucial role in the metamorphosis of the nymphs and their subsequent development into adult male and female ticks.

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Symposium Tick Physiology and Metabolism
Room Cetro, Meliá Cohiba

P09

Octopamine and α -2 adrenergic-like octopamine receptor in *Ixodes ricinus* salivary glands

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The neurotransmitter octopamine, a structural and functional analogue of the vertebrate adrenaline, plays essential roles in various aspects of invertebrate physiology. Tick octopaminergic system in relation to their biology has been studied at various levels but most advanced results were related to octopamine/tyramine-like receptor-bases susceptibility or resistance to amitraz acaricide. Here using in silico approaches we identified that ticks possess two types of adrenergic-like octopamine receptors and two types of tyramine receptors. Among those an *Ixodes ricinus* α -2 adrenergic-like octopamine receptor (Oct α 2R) has been chemically synthesized and functionally tested in the heterologous expression system. Octopamine was the most potent activator of the receptor followed by adrenaline and noradrenaline, while dopamine did not show any effect. Furthermore, an antibody against octopamine revealed six pairs of neurons in the protocerebral neuronal cells in the *Ixodes* synganglion. In salivary glands octopamine-like immunoreaction has been detected in scattered patches in close association with a single myoepithelial cell visualized by an anti-beta tubulin antibody in both type II and III acini. At this point we are unable to conclude if the immunoreactions in acini belong to the octopaminergic axon terminals, however double staining with known markers for *Ixodes* salivary gland axons, the neuropeptides and invertebrate-like dopamine receptor, showed distinct staining patterns. During the *I. ricinus* feeding, the levels of *octa2r* mRNA dramatically increases in salivary glands while not in the synganglion. Our pioneer study represents a steppingstone for a deeper molecular and biochemical exploration of the octopamine physiology in tick salivary glands.

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

P10

Tick-borne pathogens detected in ticks collected from migratory birds in Sardinia, Italy

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Introduction: Several species of ticks can infest migratory birds that may act as reservoir of tick-borne pathogens dispersing ticks over short, medium, and long distances. Migratory birds can also influence the survival and distribution of ticks during stopovers or at their destination, allowing them to colonize new habitats if conditions are suitable. Information on the role of migratory birds in the circulation of vector-borne diseases are almost absent in Sardinia, Italy. The aim of this study was to increase the knowledge on the dispersal of ticks by migrating passerine that pass through the island during their migratory route from the sub-Saharan area to Northern Europe and vice versa.

M&M: Ticks were collected from October 2021 to May 2022, from migratory birds captured at the Tumbarino Bird Observatory, Asinara, Sardinia. A first tick morphological identification was subsequently confirmed by molecular analysis. Five selected pathogens of medical and veterinary importance (*Coxiella burnetii*, *Rickettsia* spp., *Babesia* spp., *Borrelia* spp., and *Chlamydia* spp.) were targeted. The genotyping of the abovementioned pathogens was then determined by sequencing.

Results:

A total of 961 ticks, 862 during the autumn season and 99 from the spring one, were collected from 349 migratory birds. During the post-reproductive period (autumn migration) most of the ticks were collected from redbreasts (67%), blackbirds (21.8%), song thrush (8%) and woodlark (2%) while, during the pre-reproductive period (spring season) most were recovered from redstarts (55%), wood warbler species (11%), European pied flycatcher (8%) and the European robin (10%). The molecular analysis highlighted that tick species from the *Ixodes* and *Hyalomma* genus were the most abundant species collected during the autumn and spring season, respectively. In both season, larvae and nymphs were the most prevalent tick with the 65% and 26% of the total. After rt-PCR the presence of *Borrelia* spp. was highlighted in 26.1% of ticks while *Rickettsia* spp. in 17.5% of ticks. Sanger sequencing of positive ticks pointed out the presence of five *Borrelia* species: *B. garinii*, *B. valaisiana*, *B. turdi*, *B. afzelii* and *B. miyamotoi*. Among *Rickettsia* species, *R. helvetica*, *R. monacensis*, *R. aeschlimannii*, *R. sibirica* and *R. raoultii* were identified. As regards the other pathogens searched, the positivity found was as follows: 2.6% for *Chlamydia* spp., 0.6% for *Coxiella* spp., and 1.9% for *Anaplasmatidae*. For these positive samples, the molecular typing was not possible as they reported a high CT value.

Discussion: The detection of *Borrelia* and *Rickettsia* species in ticks collected from migratory birds bring out the importance of birds for the maintenance and dispersal of zoonotic pathogens. Although the colonization of new tick species in a specific area requires favourable climate, vegetation, and host conditions, as well as available ecological niche, it is necessary to understand these interactions for developing strategies to monitor and control tick populations and mitigate the risk of tick-borne diseases in both human and animal populations.

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**
Room La Corona, Meliá Cohiba

P11

Identification and antigenicity of the *Babesia ovis* spherical body protein 4 (SBP4)

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Babesia ovis is the primary causative agent of acute and chronic babesiosis in small ruminants. The disease poses serious challenges to the sheep industry, leading to significant economic losses, particularly in tropical and subtropical regions worldwide. Advanced diagnostic methods and identification of vaccine candidate antigens are crucial for effective ovine babesiosis control. The SBP4 protein, initially identified in *B. bovis*, is conserved among various geographical isolates and has demonstrated excellent performance as a serological antigen in several ELISA formats for detecting bovine babesiosis. Using the *B. bovis* SBP4 as a query, BLAST searches were conducted on an uncurated *B. ovis* genome assembly, followed by PCR amplification and sequencing of a newly identified Bo-SBP4. Characterization of this novel gene and protein was carried out using bioinformatics analysis, and ELISA. The serological response to the Bo-SBP4 protein during acute infection and post-infection periods was determined in lambs experimentally infected with the *B. ovis*-Alacakaya strain. Five lambs were experimentally infected, and blood and serum samples were collected daily for the first 20 days post-infection and biweekly for one year after the acute phase. The presence of specific antibodies against rBoSbp4 was tested in serum samples, and antibodies in some experimental animals remained positive for 400 days post-infection. The Bo-SBP4 protein shows potential as a candidate for developing a novel serological test to detect *B. ovis* infection in sheep.

Keywords: *Babesia ovis*, experimental infection, ELISA, spherical body protein 4, recombinant antigen

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CUBA - 2024

11TH TTP
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**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P12

Behavior of the biological cycle of the tick *Rhipicephalus microplus* (Acari: Ixodidae) under laboratory conditions.

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ABSTRACT

The cattle tick *Rhipicephalus microplus* has been considered one of the factors that limit the advance of livestock farming in countries affected by this parasite, where temperature and humidity conditions are determining factors for the development of this tick. The objective of the study is to determine in vitro, the behavior of the biological cycle of the *R. microplus* tick, under laboratory conditions. To determine the biological parameters of the parasitic and non-parasitic phase, 3 strains of ticks were separately infested on cattle intended for their maintenance. At the end of the infestation, teleogines were collected and transferred to the laboratory, where they were washed and dried before make groups of 20 ticks selected at random, all selected teleogines were weighed and incubated at a temperature of $27\pm 1^{\circ}\text{C}$ and Relative Humidity greater than 80%. In each group the ticks were worked individually to determine the pre-oviposition period, oviposition time, daily egg laying, eggs weight and hatching percentage. In the parasitic phase it was determined that the larvae time is 3 days, metalarvae 4 days, nymph time was 4 days and metanymphs 4 more days, and young adults developed in 6 days before detaching themselves from the host at 21 days after infestation. In the non-parasitic phase, the average weight of the teleogins was determined with 0.03127 mg, ± 0.0470 , the weight of the evoo at 15 days was 0.1507 mg, ± 0.0328 , and a total evoo weight of 0.1556 mg that did not differ between them, reaching a hatching percentage of 80.6%. Oviposition lasted 22 days, averaging 3,243 eggs per tick, depositing 99.1% of their eggs 15 days after incubation.



CUBA - 2024

11TH TTP
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Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

P13

**Stablishing a national network on the surveillance of tick-borne diseases in Spain.
Preliminary results**

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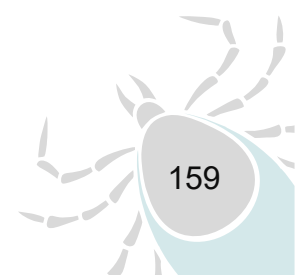
Vector-borne diseases (VBD) are of major public health and veterinary importance. In recent years, anthropogenic activities including deforestation and land use change have altered the biodiversity of ecosystems, directly contributing to the incidence of VBD. Ticks are one of these vectors who are changing their geographical distribution, favoring the dissemination of diseases in areas where they were not previously described.

This is a Spanish national network supported by the Spanish Ministry of Health (CCAES) through NGUE Funds to establish the geographical distribution patterns of different tick species and their pathogens.

In the first phase of this surveillance plan, a total of 1741 questing ticks (including larvae, nymphs and adults) were collected from vegetation in a large part of Spain. Preliminary results showed 11 different species including *Dermacentor marginatum*, *Haemaphysalis* sp., *Haemaphysalis punctata*, *Haemaphysalis sulcata*, *Hyalomma lusitanicum*, *Hyalomma* sp., *Ixodes ricinus*, *Ixodes* sp., *Rhipicephalus bursa*, *Rhipicephalus pusillus* and *Rhipicephalus sanguineus* s.l. Ticks were grouped by species in 698 pools and analyzed by molecular techniques (PCR) for the presence of viral, bacterial and parasitic pathogens. The amplified RNA/DNA of the target pathogens were then genetically characterized. Interestingly, Crimean Congo fever virus was not detected in any of the ticks (0%, 0/1684), *Rickettsia* spp. was detected in 13.1% (45/344), *Borrelia* spp. in 2.7% (10/363), *Anaplasma phagocytophilum* in 0.5% (1/194), *Babesia* spp. in 7.0% (4/57 *Ixodes ricinus*) and *Theileria* spp. in 8.7% (5/57 *Ixodes ricinus*).

At this time, we have also confirmed species such as *Rickettsia aeschlimannii* R. conorii, R. massiliae, R. monacensis, R. slovakia; *Borrelia lusitaniae*, B. valaisiana; *Babesia divergens* and *Babesia* sp. deer clade, some of them causing diseases in humans and animals.

The results of this project will be the basis for a strategic approach to controlling vectors and preventing VBD in Spain.





CUBA - 2024

11TH TTP
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**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P14

Blood pathogens in reptiles from the southeastern part of the USA

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This study focuses on researching blood pathogens in reptiles from the southeastern part of the USA. Reptiles belong to a diverse group of animals that have adapted to various types of environments. Throughout their evolutionary development, ectoparasites have co-evolved with them, potentially serving as vectors for a wide range of pathogens (viruses, bacteria, parasites). We captured 305 individuals, representing various reptile species, which were subsequently examined for the presence of intracellular or extracellular blood parasites microscopically and via molecular-genetic analyses. All capturing sites were situated in eastern part of South Carolina. Among all 279 microscopically examined samples, 17.2 % tested positive for the presence of blood parasites belonging to the phylum Apicomplexa. Among these, 47 individuals (31.8 %) were snakes (Serpentes), while only one lizard (Sauria) (0.8 %) was positive. When comparing parasitemia among different locations as well as between species, statistically significant results were observed. The average parasitemia was 27.93. Highest parasitemia was recorded in the species *Nerodia fasciata* (3504.85) and the lowest value of parasitemia in the species *Agkistrodon contortrix* (5.22). Sequencing of 18S rDNA revealed the presence of *Hepatozoon* genus in our samples. Using NGS method, we detected tick-borne bacteria belonging to genera *Rickettsia*, *Neoehrlichia*, and *Candidatus* Midichloria in blood of *Anolis carolinensis*. In the species *Eumeces laticeps*, we detected genera *Rickettsiella*, *Rickettsia*, *C. Midichloria*, *Neoehrlichia*, *Candidatus* Cryptoplasma, and *Borrelia*. Additionally, NGS method revealed the presence of *Borrelia miyamotoi* in the *E. laticeps*. Various pathogens can induce physiological and pathological changes not only in reptiles and other animals but also in humans. Therefore, understanding the relationships between vectors, pathogens, and hosts is crucial for comprehending the epidemiology and prevention of diseases associated with reptiles. The realization of this study was supported by projects APVV-19-0440, VVGS-2023-2751.



CUBA - 2024

11TH TTP
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**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P15

**Investigation of piroplasmid infection in wild and domestic birds received by fauna
enterprises in Rio de Janeiro, Brazil**

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Hemoparasites have a wide distribution and can be found in domestic and wild animals, where their infection is dependent of their vectors. This study aimed to investigate the presence of agents of the order Piroplasmida in birds both free ranging and kept under human care. 104 samples were obtained from 15 families of birds from fauna enterprises in the city of Rio de Janeiro, Brazil. Blood was collected through the medial metatarsal and right jugular vein, to search for hemoparasites by the Polymerase Chain Reaction (PCR) and blood smear technique. The samples were stored in ethylenediaminetetraacetic acid, and sent to the Laboratory for the Study of Parasite-Host Interaction (LEIPH-UFRRJ) and to the Laboratory of Hemoparasites and Vectors (LHV-UFRRJ). Only 0.96% (n=1/104) of the samples were positive in this survey. Blood smears were stained using the Giemsa method and visualized using optical microscopy, where the presence of structures similar to intraerythrocytic merozoites, suggestive of an agent from the order Piroplasmida, was observed in the sample of one *Sula leucogaster* (brown booby). For the molecular evaluation, a nested-PCR was carried out to amplify the 800 base pair fragment of the 18S rDNA gene. The amplification of the target DNA occurred in the same sample of *S. leucogaster*, corroborating the microscopic findings. The amplified product was sequenced using the Sanger method, and the sample showed 100% identity with *Babesia* sp. (KC754965), obtained from *S. leucogaster* from Brazilian islands and 99.86% similarity with *Babesia poeleana* (DQ200887) both with a coverage rate of 100%. Previous studies have demonstrated the presence of *Babesia* sp. through molecular biology in birds of the genus *Sula*, however few studies record the presence of merozoites in cytological examination. The importance of monitoring hemoparasites present in wild birds is highlighted, as their presence can cause harm to the conservation of the species.



CU3A - 2024

11TH TTP
CONFERENCE

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

P16

Molecular characterization of tick-borne infections in cattle in northern Kenya

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Abstract

Human health and animal health are closely linked in terms of the One Health concept by ticks and mosquitoes, among others, acting as vectors for zoonotic pathogens. Ticks are vectors of emerging infectious diseases that have been spreading in recent decades due to climate change and pose a threat to human and animal health. Yet, the epidemiological data on their diversity and impact on cattle in northern Kenya are limited. In this study, we characterized the diversity of ticks and tick-borne pathogens in cattle in northern Kenya using molecular tools. We screened blood and ticks (496 pools) collected from 211 cattle for presence bacterial, protozoan and viral TBPs by high-resolution melting analysis and confirmed species identity through gene sequencing. The three predominantly sampled tick species were *Rhipicephalus pulchellus* (44.2%), *Hyalomma rufipes* (32.0%), and *Amblyomma gemma* (19.5%). We detected Crimean Congo haemorrhagic fever (CCHF) virus (0.4%) in *Hy. rufipes*, and *Ehrlichia ruminantium* (8.4%), causative agent of heartwater in ruminants, in *Am. gemma* and *Rh. pulchellus* tick species infesting cattle, indicating its endemicity in northern Kenya. We also detected *Anaplasma marginale* and *Theileria velifera* in both blood and tick species, *Ehrlichia* spp., *Theileria ovis*, *Babesia* spp., *Coxiella burnetii*, *Rickettsia africae* and *Rickettsia aeschlimannii* in ticks from cattle. These findings contribute to a better understanding of the epidemiology of tick-borne diseases in cattle in northern Kenya, emphasizing the importance of surveillance and control measures to mitigate the impact of the identified TBPs on livestock health and productivity.



CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P17

***Anaplasma phagocytophilum* as a multi-host pathogen in Slovakia**

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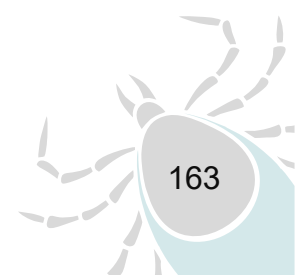
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Anaplasma phagocytophilum is an intracellular bacterium and the causative agent of granulocytic anaplasmosis of both medical and veterinary importance. In Europe, the main vector of this pathogen is *Ixodes ricinus* tick with a wide range of hosts. The objective of this study was to determine the spectrum of animal species involved in the circulation of *A. phagocytophilum* (AP) in southern Slovakia and to analyze the genetic variability of the obtained AP genotypes.

During 2011-2023, 3626 feeding *I. ricinus* ticks from various hosts (humans, dogs, cats, sheep, wild ruminants, hedgehogs, birds) were collected. 1177 questing *I. ricinus* ticks were collected by standard blanket dragging. *A. phagocytophilum* was detected in *I. ricinus* ticks by using a real-time PCR assay targeting the *msp2* gene (77bp). Positive samples were genotyped by sequencing of *msp4* and *groEL* genes. Infection prevalence in host feeding ticks was: 5.3% in ticks from humans, 17.2% from dogs, 14.4% from cats, 33.3% from sheep, 52.4% from goats, 59.7% from wild ruminants, 60% from hedgehogs and 4.6% in ticks feeding on birds. Preliminary results suggests, that prevalence of AP in questing *I. ricinus* ticks was 5.8% (Bratislava region).

From an epidemiological point of view, Cervidae and hedgehogs probably play the most important role in the circulation of this pathogen. Pets and farm animals can serve as sentinel animals in expressing the epidemiological risk for humans of being bitten by infected ticks. Overall, a wide range of animal species are involved in the maintenance and the circulation of AP in natural foci, because transovarial transmission in ixodid ticks has not been confirmed. Further genetic analyses are needed to identify the genetic variants of *A. phagocytophilum* circulating in Slovakia and their associations with reservoir hosts.

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CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P18

**MOLECULAR DETECTION OF THE HEMOTROPIC MICOPLASM IN BATS CAPTURED IN
FOREST FRAGMENTS OF PONTA GROSSA, PARANA, BRAZIL.**

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Hemotropic mycoplasmas (hemoplasmas) are globally distributed bacteria that affect domestic and wild animals as well as humans. These agents are emerging as an important object of study for bacterial pathogens in bats, but the diversity of host species studied to date is still limited. The objective of the present study was to carry out a molecular detection using a fragment of the 16S rRNA of hemotropic mycoplasma in bats captured in Ponta Grossa, State of Parana, Brazil. 19 samples of bat liver parenchyma were collected. Molecular diagnosis was performed by extracting DNA from 25 mg of liver sample using a commercial kit. An aliquot standardized to 60 ng/μL was used to amplify a 620 base pair (bp) fragment of 16S rRNA using the primers Myco 322s and Myco 938as by polymerase chain reaction (PCR). Of the 19 captured animals, seven (36.84%) amplified hemotropic mycoplasma DNA by PCR. The species of bats collected were: *Desmodus rotundus*, *Eptesicus brasiliensis*, *Lasiurus blossevillii*, *Mimon benettii*, *Myotis riparius*, *Sturnira lilium*. The distribution of the positivity for hemoplasmas in relation to the bat species was as follows: *D. rotundus* 14.2% (n=1/7), *M. benettii* 60.0% (n=3/5), *M. riparius* 100.0% (n=2/2), *S. lilium* 33.3% (n=1/3). The results of this study demonstrate the circulation of hemoplasmas in different bat species. It also highlights the importance of new studies that evaluate the genetic diversity and evolutionary distance of these pathogens in small flying mammals.

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

P19

Ticks and tick-borne pathogens in urban and forested areas in the Czech Republic

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Tick-borne bacterial diseases are among the most widespread vector-borne diseases in Central Europe, including the Czech Republic. The most common habitats for *I. ricinus* are deciduous and mixed forests and shrubs with a rich herb layer. Ticks are more common at the edges of forests, but also colonize suitable urban habitats such as parks, gardens, cemeteries, etc.

The aim of our study was to determine the abundance of ticks and their variability during the season as well as the prevalence of tick-borne pathogens in the selected urban and forested areas in the Czech Republic.

We selected an urban area of about 600 m² in three cities for intensive monitoring every month from April to December and ten urban areas for extensive testing once a year. We collected ticks from 150 forest areas on the entire territory of the Czech Republic: clear-cut areas, forests, forest edges, deciduous, coniferous and mixed forests. The average density in the urban areas reached 15.7 ticks per 100 m² and in the forest areas 12.9 ticks per 100 m².

On average, 24% of ticks from urban areas were positive for *B. burgdorferi* s. l. Other tick-borne pathogens were found in 19% of ticks; 2.3% of ticks were positive for *B. miyamotoi*, 8.4% for *A. phagocytophilum*, 1.8% for *N. mikurensis* and 6.5% for *Rickettsia* spp. The ticks from forest areas were infected with *B. burgdorferi* s.l. in 10.4% and with *A. phagocytophilum* in 1.8%.

These results show high infection rates with *B. burgdorferi* s.l. and other tick-borne pathogens in urban areas in the Czech Republic. The risks associated with exposure to ticks and tick-borne pathogens appear to be higher in urban areas than in natural ecosystems. These results add to the current knowledge on tick-borne pathogens in urban areas and highlight the potential health risk.

This study was supported by Ministry of Health of the Czech Republic, NU23-09-00049.



CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P20

**TICK INFESTING WILD AND DOMESTIC ANIMALS IN NORTHERN ITALY: TEN YEARS OF DATA
COLLECTION**

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Emilia-Romagna and Lombardia regions are located in the northern part of Italy.

Diagnosis of TBDs increased over the last years and attention of public health services focused on emerging vector borne diseases and wild fauna transmitted diseases. In this study, Ixodid ticks were collected from livestock, pets and wild animals after being hunter-killed or found dead and submitted to IZSLER laboratories for necropsy during 2013-2023, from two Italian regions (Lombardy and Emilia-Romagna). Ticks were removed and identified following Manilla taxonomic keys. A total of 14844 tick exemplars were collected from 563 municipalities in ten years of surveillance.

We sampled a total of 30 different mammal's species including humans: most sampled species are hunted wild mammals (roe deer, red deer, wild boar) due to regional surveillance plans, followed by domestic species (horse, dog and ruminants). We identified a total of 22 different tick species, belonging to 8 genera.

I. ricinus represent the main tick species, (N=7778; 52%) and it's well distributed in all northern Italy. Second more common tick species is *R. turanicus* (n=2369; 16%), followed by *R. sanguineus* (n=2226; 15%), *I. hexagonus* (n=1503; 10%), *D. marginatus* (n=411; 3%) and *H. marginatum* (n=162; 1%).

Historical data on presence/abundance of disease vectors are essential to understand TBD epidemiology and to gather data for diseases transmission. Our surveillance system allows to map geographic distribution of tick species at municipality level and provide a good base to detect and respond to vector-borne diseases related health threats.

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P21

***Borrelia burgdorferi* s.l. abundance and genetic diversity between urban greenspaces and
surrounding hinterland across the UK**

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Over 200,000 cases of Lyme disease, caused by *Borrelia burgdorferi* sensu lato, occur in Europe each year. *B. burgdorferi* s.l. is maintained in a range of wildlife reservoir hosts between which it is transmitted by *Ixodes ricinus*. As reservoir hosts and vectors are more abundant in woodland, it's widely perceived that Lyme disease is acquired in rural rather than urban settings. However, the presence of urban tick populations has been reported across Europe, occupying greenspaces that are favoured by urban planners and the public alike for the ecosystem services and well-being benefits. Given that footfall of urban greenspaces dwarves that of remoter, rural settings, the likelihood of human-tick encounter may be greater in urban settings even if tick abundance is lower. Thus, it's timely to consider the urban ecology of *B. burgdorferi* s.l. and quantify the determinants.

We hypothesize tick populations in urban greenspaces are maintained through connectivity with surrounding hinterland. Questing ticks were collected from urban and rural greenspaces in 16 towns across the UK in 2022 and 2023, and a detailed catalogue of field data from sites surveyed was recorded. Molecular methods were used to determine the presence of *B. burgdorferi* s.l. in ticks and genospecies identity. Correlations within field and laboratory data were explored using statistical modelling.

Ticks and *B. burgdorferi* were found to be more abundant in the hinterland than urban greenspaces, but we observed a correlation between the density of ticks collected at urban and hinterland sites suggesting that the two populations are indeed linked. The statistical analysis performed highlighted significant correlations between the two relating to different factors. We also encountered *B. bavariensis* in the UK and as this genospecies is considered highly pathogenic, its presence in the UK is of public health concern.



CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P22

Grey squirrels *Sciurus carolinensis*: how does an invasive species influence the *Borrelia burgdorferi* ecology in the UK?

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Grey squirrels *Sciurus carolinensis* are an invasive species in the UK and have been shown to pose threats to plants through bark stripping and other wildlife through disease and competition. As such, intense efforts are employed to control the squirrel population, traditionally involving trapping and/or shooting. Fertility control is yet to be tested for its capacity to control grey squirrel populations. Of growing concern is the potential threat grey squirrels pose to human health through their contribution as reservoir hosts to the natural maintenance of *Borrelia burgdorferi*, the agent of Lyme disease. We aim to quantify this contribution and assess the impact of different grey squirrel control methods on it. Using isolated woodlands across Cumbria, UK (n = 14), we will assess grey squirrel and tick densities before and after each culling treatment: no culling (n = 6), full cull (n = 4) and the fertility mimic cull (n = 4). Ticks will be tested for pathogen presence and blood meal analysis performed to assess the host species being fed upon. While fertility control may have a slower impact on the population density, it will alter the age ratio in the population. Given that juveniles are more mobile during dispersal, they are likely to contribute more to tick and pathogen spread than adults. Thus, fertility control may be a cost-effective method of reducing Lyme disease risk.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

P23

Genetic characterization of ticks and tick-borne parasites of selected species of captive and free-ranging felids in South Africa

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Ticks are ranked second to mosquitoes as vectors of animal and human pathogens globally. However, there are gaps in the knowledge of ticks and tick-borne pathogens in the conservation of wild animals in South Africa. Thus, this study aimed to determine the occurrence and diversity of ticks and tick-borne pathogens of selected species of wild felids using morphological keys and molecular assays. A total of 94 ticks collected opportunistically from 31 cheetahs and four lions in four game reserves in South Africa during routine health checks were identified morphologically. Blood samples were also collected from each animal. Thus, genomic DNA was extracted from 31 host blood samples (29 cheetahs & 2 lions) and the remaining 88 ticks. Additionally, 79 blood samples collected from lions (n=30), cheetahs (n=30), servals (n=16) and caracals (n=3) in different localities of South Africa were retrieved from SANBI Wildlife Biobank and included in the study. The *CO1*, *16S* and *nad5* genes for ticks, and the *18S* rRNA gene for Apicomplexan parasites (*Babesia*, *Theileria*, and *Hepatozoon* spp.) were amplified and sequenced. Nine tick species from four genera, namely *Amblyomma*, *Haemaphysalis*, *Hyalomma*, and *Rhipicephalus* were morphologically and genetically identified. *Haemaphysalis elliptica* was the most abundant species (62.8% (59/94) collected from lions and cheetahs. Parasitic infections were identified in 70.97% (22/31) of field blood samples, 23.9% (21/88) ticks, and 12.7% (10/79) of archived blood samples. BLASTn results indicate that the obtained pathogen sequences were 98 to 100% identical to *Babesia felis*, *B. lengau*, *Hepatozoon felis*, *H. luiperdjie*, published sequences and an uncharacterized *Babesia* sp. This study confirms previous reports of *Babesia* and *Hepatozoon* spp. infections in wild felids in South Africa and further highlights the importance of monitoring these infections in wild animals prior to translocation to prevent transference of ticks and the transmission of pathogens to new hosts and localities.

Keywords: Felids, ticks, *CO1*, *16S*, *nad5*, piroplasms, *18S*, South Africa



CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P24

Patterns of cattle breed sensitivity to the tick *Rhipicephalus microplus*

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Keywords: Tick Ruler, *Rhipicephalus microplus*, resistance, host

Abstract

Among cattle, *Bos taurus* breeds and their crosses are more sensitive to tick infestations; in contrast, *Bos indicus* breeds are more resistant to infestation and more adaptable to tropical climates. Herds with susceptible breeds and inadequate tick control generate direct and indirect losses in the meat production chain, in addition to increased mortality due to cattle tick fever (CTF). The objective of this study was to describe, compare and rank the sensitivity of different breeds of stabled cattle to the tick *Rhipicephalus microplus* and to present, as an innovative result, a scale called the Tick Ruler. Secondary data on the number of retrieved engorged females, engorged female ticks' weight, egg mass weight and number of larvae were extracted from research reports of experiments conducted over 18 years with eight breeds to describe and report the sensitivity of breeds to artificial infestation of *R. microplus* larvae. For analyses, the recovery rate of engorged female ticks (RREF), the percentile of dispersion of individuals in their respective races, and the comparison of these percentiles between races were calculated. The ranking of the percentiles resulted in the organization of the breeds by their susceptibility to *R. microplus*; we call this scale the "Tick Ruler." The ruler is a simple, easy-to-understand tool that can be used by technicians and producers to evaluate the position of the breed of interest in relation to tick sensitivity and can assist producers in decision-making to find a balance between increased production gains and the risk of economic losses depending on the breed composition in the cattle herd.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

P25

Investigating tick-borne pathogens in questing and potential reservoir ticks in Portugal

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Ticks are ubiquitous hematophages ectoparasites with the ability to transmit bacteria, viruses and protozoan pathogens to their vertebrate host. Neglected infections caused by a large spectrum of tick-borne pathogens (TBP) have become a global public health concern, especially when these TBP cause zoonoses. Ixodid ticks are the main vectors of TBP and may be reservoirs of many pathogen species that can be vertically transmitted. Thus, their characterization is essential to assess the risk of disease and improve control measures. The present study aimed to identify TBP infecting questing ticks in mainland Portugal. From May 2019 until May 2021, questing ticks (females, males, and nymphs) were collected from the vegetation by dragging method at five different ecological sites in mainland Portugal. Ticks collected were identified using taxonomic keys and submitted to DNA and RNA extraction. The absence of PCR inhibitors was confirmed by screening DNA samples for the presence of the tick 18SrRNA gene. Samples were screened for the presence of *Rickettsia* spp. by TaqMan real-time (qPCR), targeting a 74-bp fragment of the citrate synthase gene (gltA). Positive samples were subsequently submitted to conventional PCR targeting larger fragments of the gltA and ompA (outer membrane protein) rickettsial genes. Universal SYBR qPCR was performed to amplify a 107-bp fragment of the 16S rRNA gene to detect *N. mikurensis*. Conventional PCR assay was performed to amplify both a 408-bp fragment of the 18SrDNA gene, and a 345-bp fragment of the 16SrRNA gene of piroplasmids and Anaplasmatacea bacteria, respectively. A total of 802 questing ticks were collected. *Ixodes ricinus* was the most prevalent tick species (n=619/77.18%). Phylogenetic analysis has shown *Babesia bigemina*, *Babesia* sp., *Theileria* sp., *Rickettsia helvetica*, *Rickettsia massiliae*, *Rickettsia monacensis*, *Rickettsia slovacica* and *Candidatus Rickettsia rioja* infecting questing ticks from four ecological sites. The outcome of this study contributes to a better understanding of TBP prevalence from questing ticks collected in mainland Portugal.



CUBA - 2024

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

P26

***Anaplasma phagocytophilum* in passerines from the Ile-de-France region (France), genetic characterization of variants by *groEL*, *ankA* and MLST typing**

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Anaplasma phagocytophilum (*Aph*) is a strict intracellular bacterium transmitted by ticks of the genus *Ixodes*. It causes granulocytic anaplasmosis (GA) in humans and various species of domestic mammals (dogs, cattle, sheep...) also called tick-borne fever (TBF) in ruminants. Contrary to TBF which is particularly prevalent in Europe and is the cause of significant economic losses, human GA is mainly found in the USA. *Aph* is characterized by high genetic diversity and complex epidemiological cycles, as it is also found in many wild animals (deer, rodents, birds...). Domestic species such as horses and dogs, as well as wild species such as wild boar, foxes and hedgehogs, harbor strains that are suspected of being zoonotic. Conversely, deer, rodents (voles) and shrews, as well as birds apparently, appear to harbor strains that are genetically distant from those that affect humans. Birds play an important role in the maintenance and spread of pathogens. *Aph* DNA has been identified in avian hosts in Europe, with prevalence rates ranging from 0% to 33.8%. However, few epidemiologic studies have been conducted on birds and *Aph*. Our project therefore aimed to investigating the presence of *Aph* in birds from urban and peri-urban areas of Ile-de-France and neighboring departments, particularly in passerines. Liver, spleen and skin samples from 680 passerines were tested for the presence of *Aph* DNA by qPCR targeting the *msp2* gene. Three blackbirds (*Turdus merula*) were tested positive for *Aph* DNA. This corresponds to a detection rate of 0.4 % in all the birds tested and 3.3 % in the blackbirds. In a second phase, a phylogenetic study was performed targeting the *groEL* and *ankA* genes and using MLST (multilocus sequence typing). These results support the existence of an epidemiological cycle specific to birds, in France, in agreement with what is found in other European countries.

Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens
Room La Corona, Meliá Cohiba

P27

Population genomics of *Borrelia burgdorferi* sensu lato in Italy

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Borrelia burgdorferi sensu lato is a bacterial complex that include all spirochete responsible for Lyme disease, the tick-borne disease with the highest incidence in Europe and North America. Lyme disease is characterised by an early stage with initial symptoms ranging from malaise, fatigue, headache, arthralgia, myalgias and fever and can then develop into a chronic and debilitating illness characterised by skin lesions, neurological disorders, carditis and arthritis. In Europe, the most commonly reported genospecies are *B. afzelii* and *B. garinii*, which are transmitted by the tick vector *Ixodes ricinus*.

Despite its clinical importance, limited knowledge is available about the genomics of this pathogen, mostly due to the highly fragmented architecture of its genome. Indeed, short read sequencing technologies have been applied to small and large datasets but, as expected, have proven not to be fully adequate to assemble the numerous plasmids. Since there are currently no Italian genomes available, our first aim is to isolate *B. burgdorferi* s.l. from ticks and patients in Northern Italy, and then to obtain high-quality genomes by exploiting both short- and long-read sequencing technologies. Since it has been suggested that different genospecies and genotypes can give rise to different clinical manifestation, our second goal is to investigate the presence of genetic markers responsible for infection and pathogenesis in humans.

So far, we obtained six genomes of isolates obtained from single ticks collected in Italy, five *B. garinii* and one *B. burgdorferi* s.s. and we performed preliminary bioinformatic analyses. We obtained genomic-based phylogenies and applied a method to compare plasmids of the newly sequenced isolated to those retrieved from public databases. Our results show a limited level of species-specificity in the plasmid contents. Ongoing work is underway for the isolation and sequencing of other *Borrelia* cultures and for more advanced bioinformatic analyses.



CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P28

**Molecular investigation of hemoparasites of the order Piroplasmida in capybaras from
endemic and non-endemic areas for Brazilian Spotted Fever in Brazil**

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The population of capybaras (*Hydrochoerus hydrochaeris*) in Brazil is growing, increasing their presence in urban parks and their contact with humans. Known for their role in the transmission of Brazilian Spotted Fever, capybaras may be affected by other diseases with zoonotic potential that, combined with their population growth, pose a public health problem. Although the zoonotic *Babesia spp.* is not considered endemic in South America and is not related to wildlife, studies indicate the possible involvement of capybaras and associated ticks, such as *Amblyomma sculptum* and *Amblyomma dubitatum*, in the life cycle of piroplasmids. The present study aims to carry out an epidemiomolecular investigation of the piroplasmids present in capybaras in endemic and non-endemic areas for Brazilian Spotted Fever in the interior of São Paulo State and the Brazilian Pantanal in Mato Grosso do Sul and Mato Grosso, respectively. 155 blood samples and DNA were collected using a commercially available kit. To detect piroplasmids, the samples were subjected to PCR, using primers BAB2 143-167 and BAB2 694-667 (551bp), corresponding to the conserved regions of the 18S rRNA gene of *Babesia*, *Theileria* and *Cythauxzoon*. The amplification products were visualized by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and then analyzed for the presence and absence of bands. Of the 155 samples, 93 were positive for hemoparasites of the order Piroplasmida, with the following geographical distribution 10 samples in the city of Americana/SP, 7 in Araras/SP, 6 in Avaré/SP, 14 in Piracicaba/SP, 7 in Pirassununga/SP, 12 in Ribeirão Preto/SP, 11 in Tatuí/SP, 16 in Corumbá/MS and 10 in Poconé/MT. This is a preliminary study and a larger fragment of 18S rDNA will be amplified to characterize and identify the piroplasm to species level.

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P29

**VECTOR ABUNDANCE AND ASSOCIATED ABIOTIC FACTORS THAT INFLUENCE THE
DISTRIBUTION OF TICKS IN SIX PROVINCES OF SOUTH AFRICA**

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Ticks are recognized as significant arthropod parasites due to their ability to thrive across diverse climates and hosts. Their distribution patterns are intricately influenced by various factors such as climate conditions, and host densities. Specifically, hard ticks are the most diverse group within the Ixodida order reported globally. As climate change continues to alter environmental conditions, it inevitably impacts the presence and distribution of ticks, consequently affecting the prevalence of tick-borne diseases. Therefore, there is an urgent need for comprehensive research aimed at understanding the factors contributing to the dispersal of ticks from cattle. The study involved the collection of hard ticks from communal cattle across six provinces in South Africa. Cattle were brought to dip tanks for tick collection and control. Climate data was obtained from an online source and analyzed to identify key patterns. Tick sampling involved careful removal and preservation of ticks. Tick counting was conducted with tick species being grouped and sorted according to physical similarity and location. Statistical analysis was performed using IBM SPSS Statistics 26.0, including descriptive statistics and Chi-square tests to assess associations between tick species presence and regions, as well as ANOVA to compare mean tick counts among regions. The study revealed distinct geographic variations in tick distribution, with KwaZulu-Natal exhibiting the highest tick count (44.5%), followed by Limpopo (38.2%), and the Eastern Cape (18.3%). *Amblyomma hebraeum* (54.4%) and *R. evertsi evertsi* (14.5%) emerged as the dominant species across all regions, while other species displayed localized distribution patterns. Chi-square tests indicated significant associations between tick species presence and regional factors. Analysis of climate data showed correlations between tick infestation and factors such as temperature, humidity, and rainfall. Regions with higher temperatures and humidity levels exhibited increased tick abundance, emphasizing the role of climatic conditions in tick infestation patterns.



CUBA - 2024

11TH TTP
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**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P30

**New patterns in seasonal activity of two most common ticks in Slovakia *Ixodes ricinus* and
Dermacentor reticulatus and its infection with tick-borne agents**

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Ticks transmit a wide range of pathogenic microorganisms. Many of tick-borne diseases have emerged (or re-emerged) within the past 2 or 3 decades. New foci of tick-borne diseases can be formed due to the climatic changes.

We focused on the study of changes in the distribution and seasonal activity of two epidemiologically important species, *Ixodes ricinus* and *Dermacentor reticulatus*.

Seasonal activity was monitored in Bratislava since June 2023. Ticks have been collected monthly by flagging the vegetation. Moreover, we are monitoring the activity of ticks directly using tick gardens.

The first results indicate a significant changes in the seasonal activity of ticks compared to the situation in the past. The highest number of *I. ricinus* ticks was recorded in June (n = 550) and in November (n = 2286) where larvae predominated (97.6%). In August, the questing activity of ticks significantly decreased (n = 8). In fall (September and October) a few nymphs and adult ticks were collected (n = 64) and larvae predominated (86.3%). Ticks, mainly larvae (n = 73) were still active during the warm December (above 10 °C). Similar situation was recorded in January, when 98 ticks were flagged including 96.9% of larvae. In February and March, the number of nymphs and adults increased to 91 and 96, respectively. The number of larvae has decreased to 6 and 0, respectively.

Questing activity of *D. reticulatus* in tick gardens was observed even at sub-zero temperatures and also during the snow cover (December and January).

Study of changes in questing activity of ticks, and the prevalence, as well as genetic variability of tick-borne pathogenic agents, will show us a picture of the current risk of infection with tick-borne diseases.

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CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P31

The role of birds in the dispersal of ticks and other arthropod vectors

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During their transcontinental migration, wild birds have long been recognized as carriers of ticks and other arthropods. These parasites frequently serve as vectors for pathogens that pose potential risks to both humans and animals. In Central Hungary, the monitoring of wild birds in this regard has been constant in the previous decade, and has since been expanded to encompass multiple other locations within the country. During our work, hard ticks (Ixodidae) and louse flies (Hippoboscidae) were removed from passerine birds and were identified by both morphological and molecular methods. Due to the distinctive ecological characteristics of the Carpathian Basin and the comprehensive scope of our sample collection efforts, we have successfully identified ten distinct species of ticks (*Ixodes ricinus*, *Haemaphysalis concinna*, *Ixodes frontalis*, *Haemaphysalis punctata*, *Ixodes lividus*, *Ixodes festai*, *Ixodes arboricola*, *Hyalomma rufipes*, *Hyalomma marginatum*, and *Dermacentor reticulatus*) from birds. Our findings indicate significant differences in habitat, migration patterns, and feeding heights of the avian hosts of the two most abundant tick species (*Ixodes ricinus* and *Ha. concinna*). In 2022, a total of twelve subadults of an African tick species, *Hy. rufipes* were gathered from avian hosts across various sites in Western and Southern Hungary. Among these, seven specimens were retrieved from Bearded Reedlings (*Panurus biarmicus*), a resident bird species, during its nesting period in the summer, at a single locality (South-Western Hungary). This comprised six nymphs and one unfed larva, that belonged to the same haplotype according to three different mitochondrial genetic markers. These data were considered as strong evidence of the first European reproduction of *Hyalomma rufipes*. However, the persistence of the population in the area has not yet been verified. The first European appearance of the African louse fly, *Ornithoctona laticornis* was also revealed.

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**
Room La Corona, Meliá Cohiba

P32

Citizen science monitoring of tick contact areas in Finland – Lessons, results and uses for data

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Ticks and tick-borne diseases (TBDs) form a significant and growing threat to human health globally. In the absence of vaccines against TBDs other than TBE, or effective ways for broad scale controlling of tick population sizes, increasing public awareness regarding tick risk areas, biotopes and activity seasons remains the primary method for preventing TBDs. In order to form comprehensive and up-to-date maps of tick occurrence and, consequently, tick risk areas, vast networks of researchers or citizen science are required. Citizen science, sometimes referred to as crowdsourcing, is an efficient method for collecting data on invertebrate vectors over broad spatial scales.

The University of Turku tick project (www.puutiaiset.fi) has conducted two nationwide crowdsourcing campaigns in Finland to map tick contact areas and collect tick samples. In 2015, we organized a nationwide tick collection campaign to assess the distribution ranges of *Ixodes ricinus*, *I. persulcatus*, receiving ~20 000 ticks in 7000 letters during the summer. In 2021, we launched an online website for reporting tick observations and observing risk areas, Punkkilive (www.punkkilive.fi/en). Punkkilive has received over 300 000 tick observations since its launch in April 2021.

In this presentation, I will share results from our nationwide crowdsourcing studies. I will show that ticks are now contacted approximately 400 km further north in the western parts and 100 km further north in the eastern parts of the country than in the 1950's. However, I will also show that it is not likely the native species, *I. ricinus*, which is behind the majority of the northwards expansion in tick contact areas, but rather the invasive species *I. persulcatus*. Finally, I will share our observations regarding the spatial occurrence and varying strength of sampling bias in our crowdsourced data set, as well as some creative uses we have discovered for the data.

**Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens**

Room La Corona, Meliá Cohiba

P33

Vector role of *Hyalomma* ticks: Comparison of prevalence of infection between ticks collected from two CCHFV-endemic countries (Mongolia and Iraq)

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Ticks of genus *Hyalomma*, especially of the *H. marginatum* complex, are known as vectors of Crimean-Congo hemorrhagic fever virus (CCHFV). *Hyalomma* spp. are thermophilic species inhabiting mainly dry and warm habitats (deserts, semi-deserts, pastures) of southern Europe, Middle East, Africa and Asia. Occurrence of *Hyalomma* ticks is associated with a presence of CCHFV, which is one of the most genetically diverse representatives of the family *Nairoviridae*.

The main goal of this study is to compare prevalence of CCHFV infection in two CCHFV-endemic countries, Iraq and Mongolia, including both known endemic areas and new locations.

Ticks were collected during three expeditions: in Mongolia in summer 2023 and spring 2024, and in Iraq in spring 2024. We aimed to collect and examine *H. anatolicum* and *H. dromedarii* in Iraq and *H. asiaticum* in Mongolia.

For the detection of CCHFV in ticks one-step real-time RT-PCR assay was designed. The novel technique is based on the *Taq*-man probes technology employing the endonuclease activity of *Taq* polymerase enzyme.

During the conference, results regarding a comparison of CCHFV prevalence in *Hyalomma* ticks from two CCHFV-endemic countries will be presented as well as a description of modern molecular techniques used to detect the virus in ticks.

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Symposium Ecology and epidemiology of Ticks and
tick-borne Pathogens

Room La Corona, Meliá Cohiba

P34

**First detection of *Ixodiphagus hookeri* (Hymenoptera: Encyrtidae) in *Ixodes ricinus* ticks
(Acari: Ixodidae) in Hungary**

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The parasitoid wasp *Ixodiphagus hookeri* (Hymenoptera: Encyrtidae) is a known natural enemy of various hard and soft tick species, including *Ixodes ricinus* (Acari: Ixodidae). Although encyrtid wasps are believed to have a worldwide distribution, there are limited studies detailing their actual global presence. Our research aimed to fill this gap by investigating the distribution of *I. hookeri* in Central Europe, specifically in Hungary. The shotgun metagenomic sequencing-based analysis included the study of bacterial patterns that potentially represent parasitoids as well.

In addition to introducing an indirect, bacterial-pattern-based metagenomic approach for identifying potential parasitoids, this study marks the first documented occurrence of *I. hookeri* in Hungary, thereby extending the known range of this species to the southernmost region of Central Europe. We collected and analyzed *I. ricinus* nymphs from multiple sampling points across Hungary. *I. hookeri* infestations were detected at five distinct locations, confirming the presence of this parasitoid wasp in the region.

The findings underscore the limited understanding of *I. hookeri*'s precise distribution. Given the emerging public health challenges posed by climate change, there is a pressing need to explore the potential of *I. hookeri* as a biological control agent for tick populations. Additionally, the role of *I. hookeri* as an ecological bioindicator warrants further investigation. Addressing these gaps in knowledge is crucial for developing integrated pest management strategies and understanding the broader ecological implications of parasitoid wasps in tick population dynamics.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Ecology and epidemiology of Ticks and tick-borne Pathogens

Room La Corona, Meliá Cohiba

P35

Tick-borne pathogens detected in ticks collected from migratory birds in Sardinia, Italy

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Introduction: Several species of ticks can infest migratory birds that may act as reservoir of tick-borne pathogens dispersing ticks over short, medium, and long distances. Migratory birds can also influence the survival and distribution of ticks during stopovers or at their destination, allowing them to colonize new habitats if conditions are suitable. Information on the role of migratory birds in the circulation of vector-borne diseases are almost absent in Sardinia, Italy. The aim of this study was to increase the knowledge on the dispersal of ticks by migrating passerine that pass through the island during their migratory route from the sub-Saharan area to Northern Europe and vice versa.

M&M: Ticks were collected from October 2021 to May 2022, from migratory birds captured at the Tumbarino Bird Observatory, Asinara, Sardinia. A first tick morphological identification was subsequently confirmed by molecular analysis. Five selected pathogens of medical and veterinary importance (*Coxiella burnetii*, *Rickettsia* spp., *Babesia* spp., *Borrelia* spp., and *Chlamydia* spp.) were targeted. The genotyping of the abovementioned pathogens was then determined by sequencing.

Results:

A total of 961 ticks, 862 during the autumn season and 99 from the spring one, were collected from 349 migratory birds. During the post-reproductive period (autumn migration) most of the ticks were collected from redbreasts (67%), blackbirds (21.8%), song thrush (8%) and woodlark (2%) while, during the pre-reproductive period (spring season) most were recovered from redstarts (55%), wood warbler species (11%), European pied flycatcher (8%) and the European robin (10%). The molecular analysis highlighted that tick species from the *Ixodes* and *Hyalomma* genus were the most abundant species collected during the autumn and spring season, respectively. In both season, larvae and nymphs were the most prevalent tick with the 65% and 26% of the total. After rt-PCR the presence of *Borrelia* spp. was highlighted in 26.1% of ticks while *Rickettsia* spp. in 17.5% of ticks. Sanger sequencing of positive ticks pointed out the presence of five *Borrelia* species: *B. garinii*, *B. valaisiana*, *B. turdi*, *B. afzelii* and *B. miyamotoi*. Among *Rickettsia* species, *R. helvetica*, *R. monacensis*, *R. aeschlimannii*, *R. sibirica* and *R. raoultii* were identified. As regards the other pathogens searched, the positivity found was as follows: 2.6% for *Chlamydia* spp., 0.6% for *Coxiella* spp., and 1.9% for *Anaplasmatidae*. For these positive samples, the molecular typing was not possible as they reported a high CT value.

Discussion: The detection of *Borrelia* and *Rickettsia* species in ticks collected from migratory birds bring out the importance of birds for the maintenance and dispersal of zoonotic pathogens. Although the colonization of new tick species in a specific area requires favourable climate, vegetation, and host conditions, as well as available ecological niche, it is necessary to understand these interactions for developing strategies to monitor and control tick populations and mitigate the risk of tick-borne diseases in both human and animal populations.

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

P36

***Babesia bigemina* enolase: a plasminogen-binding protein that enhances plasminogen activation and induces neutralizing antibodies to the infection of *in vitro* erythrocyte culture**

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RESUMEN.

Babesia bigemina is a vector-borne pathogen that causes important economic losses in livestock in Mexico and other countries. Enolase is a glycolytic enzyme with multiple functions. In many vector-borne pathogens this enzyme plays an important role in the process of invasion, serving as a surface receptor for the binding of plasminogen on a variety of cells surfaces. The plasminogen is converted to plasmin, aiding in the degradation of the fibrin of the extracellular matrix and facilitating the pathogen invasion process. *Babesia* parasites have a complex life-cycle, including asexual stages in the bovine host and sexual stages in ticks and little is known about the molecular events and proteins that lead to tick midgut invasion. In this study we confirmed that the recombinant enolase of *B. bigemina* (rEnoBb) binds plasminogen through ligand blotting assay and the binding is inhibited by the epsilon-aminocaproic acid (EACA), a lysine analogue. Moreover, the binding of rEnoBb to plasminogen showed an increase of the Plasminogen activation by tPA. Besides specific anti-rEnoB significantly neutralized erythrocyte invasion into *in vitro* *B. bigemina*. Based on our results, we can hypothesize that *Babesia bigemina* enolase could be playing an important role facilitating pathogen invasion of erythrocytes.

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

P37

Norwegian distributional changes of Tick-borne encephalitis virus in a nutshell

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The zoonotic flavivirus tick-borne encephalitis virus (TBEV) is endemic in southern Norway which is transmitted to humans infected *Ixodes ricinus* tick bites. The purpose of this study was to present updated overview of the changes in the distribution of TBE cases along the southern coastline from 2009 until present.

Between 2009 – 2015, the highest estimated TBEV prevalence in ticks was detected in Rogaland (southwestern coast) and Vestfold (southeastern coast) Counties with 5,3% and 5,5%, respectively. Especially after 2018, an annual increase of TBE cases is reported by the National Surveillance System for Communicable Diseases (MSIS). Notably, they seem to be more widely distributed in Norway than previously anticipated and the distribution has expanded to new areas in the northern, western, and eastern direction. This could be related to climatic changes and new transmission sites. There is a time lag between the previous detection of TBEV in ticks and the recent appearance of cases from the same sites. Surveillance and risk evaluation of TBEV is an important part of the national reference function.

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

P38

Evaluation of tick-borne parasites in blood smears of wildlife collected during an anthrax outbreak in the Kruger National Park, South Africa

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Southern African wildlife are known to host a wide variety of tick species of veterinary and medical importance. A routine passive disease surveillance programme was implemented in Kruger National Park, South Africa, primarily to monitor anthrax outbreaks, but which is a rich source of data and archival blood smears. Given that wildlife are considered to be sentinels of tick-borne protozoans and select bacterial pathogens of veterinary importance, the aims of this retrospective study were to i) evaluate the blood smears and accompanying robust database as a resource for vector-borne disease investigations and ii) investigate infections caused by *B. anthracis* and intracellular parasites from smears collected in 2010 during anthrax outbreaks.

Blood smears (n=100) collected from animal carcasses during anthrax outbreaks in 2010 were evaluated by microscopic, bacteriological and molecular techniques. Of the two slides collected per carcass, one was Giemsa stained for microscopic examination and used to visually score the slide according to the level of decomposition, smear thickness and presence of pathogens. The blood, from the remaining unstained slide, was scraped off and divided into two aliquots. The first aliquot was used for bacteriological culture while the second aliquot was subjected to a DNA extraction protocol. The DNA was used in a diagnostic qPCR targeting *Bacillus anthracis* and intracellular parasites. All samples were similarly tested for intracellular parasites, such as piroplasms, using reverse line blot (RLB) assay and real-time PCR.

In this sample set, 96% of the animals (n=96/100) succumbed to anthrax. More importantly, we report on co-infections in these animals by haemoparasites (n=44), from RLB. This study demonstrates the value of archival smears in providing a snapshot of the infection status of animals to intracellular and bacterial pathogens. As baseline data, it has the potential to be coupled with tick distribution, tick burden and climate change data to inform infection dynamics in wildlife species.



CUBA - 2024

11TH TTP
CONFERENCE

Pathogenesis of ticks and tick-borne pathogens

Room Internacional II, Meliá Internacional Varadero

P39

**ASSESSMENT OF TICK DIVERSITY AND POTENTIAL PATHOGEN TRANSMISSION IN TWO
ECOLOGICAL NICHES: IMPLICATIONS FOR ZOO NOTIC DISEASE SURVEILLANCE**

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Ticks play a significant role in disease transmission, rivaling mosquitoes in importance, yet knowledge gaps persist regarding ticks of public health importance. Their remarkable diversity stems from complex life cycles and interactions with hosts, leading to their ubiquity across diverse animal species. This study aimed to assess tick diversity in the Ghanaian Shai Hills Resource Reserve and surrounding communities. Tick specimens were collected from various hosts, including wild animals like *Struthio camelus*, *Python regius*, and *Equus quagga*, and domestic animals like *Bos taurus*, *Canis lupus familiaris*, *Felis catus*, and *Ovis aries*. *Amblyomma variegatum* emerged as the predominant tick species on domestic hosts outside the reserve, while *Rhipicephalus evertsi* prevailed among ticks from captive wild animals within the reserve. *Amblyomma variegatum*, particularly prevalent on *Bos taurus*, which freely moves and forages in the reserve's buffer zones, was observed across both study sites. Given its association with zoonotic diseases like Crimean-Congo hemorrhagic fever and African tick-bite fever, the presence of *Amblyomma variegatum* underscores the risk of pathogen transmission. Molecular analysis targeting the cox-1 gene confirmed tick species identification. The findings emphasize the importance of surveillance and management strategies to mitigate the public health risks posed by ticks and the pathogens they carry, urging further investigation into the specific pathogens transmitted by these vectors.

Keywords: Ticks, *Amblyomma variegatum*, Resource reserve, *Rhipicephalus evertsi*, *Bos taurus*

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

P40

Borreliosis on the Crimean Peninsula

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In Russia, borreliosis by ixodid ticks occupies on the first places and in the Crimea. The environmental the change of existence of ixodids is expanding, detection other zoonanthroponoses: ehrlichiosis, human granulocytic anaplasmosis, rickettsia. In ticks removed from humans was detected the tick-borne encephalitis virus, rickettsiae and *Borrelia burgdorferi* sensu lato (s.l.) complex: *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto. In our study of 257 *I. ricinus* ticks collected on the territory of the peninsula, we found *B. miyamotoi* in 1.7%. The level of infection of ticks with *B. miyamotoi* is lower than

B. burgdorferi s.l. (5.4%). The risk zone includes the central and southern regions of the Crimea, located in mountain forest, foothill and forest-steppe landscapes, including the cities of Feodosia and Yalta. In 2017, for the first time, markers of tick-borne borreliosis were identified in small mammals (small shrew, wood mouse, etc.) captured in the steppe zone of the republic, which was not previously considered endemic for borreliosis.

The clinical of tick-borne relapsing fever has differences in borreliosis caused by *B. miyamotoi*: at the site of a tick bite, there is almost no primary affect, no erythema, the disease begins acutely with a sharp chill and an increase in temperature to 38–40 ° C. More pronounced intoxication, febrile and catarrhal syndromes and elevation of transaminases, leuko-, thrombocytopenia. Often, the clinical manifestations of infection caused by *B. miyamotoi* (tick-borne relapsing fever) coincide with the manifestations of tick-borne encephalitis.

The main sources and vectors of *B. miyamotoi* on the Crimea are not fully understood. the atypical picture of tick-borne borreliosis may be a reflection of undetected *B. miyamotoi* pathogens in patients. Incomplete and untimely diagnosis of *B. miyamotoi* may be the cause of inadequate therapy, chronic infection and disability of patients.



CUBA - 2024

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Pathogenesis of ticks and tick-borne pathogens

Room Internacional II, Meliá Internacional Varadero

P41

Tick-borne pathogens detected in ticks collected from migratory birds in Sardinia, Italy

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Introduction: Several species of ticks can infest migratory birds that may act as reservoir of tick-borne pathogens dispersing ticks over short, medium, and long distances. Migratory birds can also influence the survival and distribution of ticks during stopovers or at their destination, allowing them to colonize new habitats if conditions are suitable. Information on the role of migratory birds in the circulation of vector-borne diseases are almost absent in Sardinia, Italy. The aim of this study was to increase the knowledge on the dispersal of ticks by migrating passerine that pass through the island during their migratory route from the sub-Saharan area to Northern Europe and vice versa.

M&M: Ticks were collected from October 2021 to May 2022, from migratory birds captured at the Tumbarino Bird Observatory, Asinara, Sardinia. A first tick morphological identification was subsequently confirmed by molecular analysis. Five selected pathogens of medical and veterinary importance (*Coxiella burnetii*, *Rickettsia* spp., *Babesia* spp., *Borrelia* spp., and *Chlamydia* spp.) were targeted. The genotyping of the abovementioned pathogens was then determined by sequencing.

Results:

A total of 961 ticks, 862 during the autumn season and 99 from the spring one, were collected from 349 migratory birds. During the post-reproductive period (autumn migration) most of the ticks were collected from redbreasts (67%), blackbirds (21.8%), song thrush (8%) and woodlark (2%) while, during the pre-reproductive period (spring season) most were recovered from redstarts (55%), wood warbler species (11%), European pied flycatcher (8%) and the European robin (10%). The molecular analysis highlighted that tick species from the *Ixodes* and *Hyalomma* genus were the most abundant species collected during the autumn and spring season, respectively. In both season, larvae and nymphs were the most prevalent tick with the 65% and 26% of the total. After rt-PCR the presence of *Borrelia* spp. was highlighted in 26.1% of ticks while *Rickettsia* spp. in 17.5% of ticks. Sanger sequencing of positive ticks pointed out the presence of five *Borrelia* species: *B. garinii*, *B. valaisiana*, *B. turdi*, *B. afzelii* and *B. miyamotoi*. Among *Rickettsia* species, *R. helvetica*, *R. monacensis*, *R. aeschlimannii*, *R. sibirica* and *R. raoultii* were identified. As regards the other pathogens searched, the positivity found was as follows: 2.6% for *Chlamydia* spp., 0.6% for *Coxiella* spp., and 1.9% for *Anaplasmatidae*. For these positive samples, the molecular typing was not possible as they reported a high CT value.

Discussion: The detection of *Borrelia* and *Rickettsia* species in ticks collected from migratory birds bring out the importance of birds for the maintenance and dispersal of zoonotic pathogens. Although the colonization of new tick species in a specific area requires favourable climate, vegetation, and host conditions, as well as available ecological niche, it is necessary to understand these interactions for developing strategies to monitor and control tick populations and mitigate the risk of tick-borne diseases in both human and animal populations.

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

P42

TBEV interaction with human microglia

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Tick-borne encephalitis virus (TBEV) is a neurotropic orthoflavivirus known to cause severe infections of the central nervous system associated with extensive neuroinflammation. However, the exact mechanism by which TBEV damages the central nervous system remains unclear. While neurons are recognized as primary targets, the involvement of microglia, a key component of the brain's immune response, remains unclear. This ambiguity extends to other orthoflaviviral infections and raises the question of whether microglia exert a protective or damaging effect.

In this study, we sought to clarify the susceptibility of human microglia to TBEV and to investigate the resulting immune response in different viral strains. Primary human microglia and two immortalized human microglial cell lines were exposed to three TBEV strains — Hypr, Neudörfl and 280 — each with different levels of virulence. Using plaque assays and genomic vRNA quantification, we followed the dynamics of viral replication. We also used Luminex multiplex assays to measure cytokine/chemokine production in primary human microglia following infection with different viral strains. Using immunocytochemistry and transmission electron microscopy, we were able to visualize the distribution of viral antigens, assess the susceptibility of microglia to infection and investigate the ultrastructural changes caused by TBEV.

Our results show that all human microglia cultures tested were susceptible to TBEV and maintained long-term productive infections in all viral strains. Remarkably, microglial responses differed depending on the viral strain and affected the dynamics of secreted modulating factors such as IP-10, MCP-1, IL-8 and IL-6. The highly virulent strain elicited the highest production of these factors, while unexpectedly similar levels were observed in the avirulent strain. Conversely, the Neudörfl strain was associated with the lowest production. Although TBEV infection did not induce cytopathic effects, electron microscopy revealed extensive changes in the ultrastructure of the infected cells.

Our study highlights the susceptibility of human microglial cells to TBEV infection and describes strain-dependent variations in viral replication dynamics and cytokine/chemokine production. These results shed light on the intricate interplay between microglia and TBEV and contribute to our understanding of TBE pathogenesis and neuroinflammation.

Pathogenesis of ticks and tick-borne pathogens
Room Internacional II, Meliá Internacional Varadero

P43

A shared pathogen: *Babesia rossi* in domestic dogs, black-backed jackals (*Canis mesomelas*) and African wild dogs (*Lycaon pictus*) in South Africa

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Keywords: African wild dog, *Babesia rossi*, Black-backed jackal, *Canis mesomelas*, *Lycaon pictus*, Reservoir host, South Africa

A B S T R A C T

In sub-Saharan Africa, babesiosis in domestic dogs is caused primarily by *Babesia rossi*. Black-backed jackals (*Canis mesomelas*), which are subclinical carriers of *B. rossi*, were a likely reservoir host from which infection passed to domestic dogs. The role of other indigenous canids, e.g. African wild dogs (*Lycaon pictus*), as reservoirs of *B. rossi* has not been elucidated. The question also arises whether genetic differences have arisen between *B. rossi* infecting domestic dogs and “ancestral” *B. rossi* in jackals. In a previous study we found that nearly one third (27 of 91) of jackals were infected with *B. rossi*; this was confirmed by 18S rDNA sequence analysis. In this study, the near full-length *B. rossi* 18S rRNA gene was successfully amplified from 6 domestic dogs and 3 black backed jackals. The obtained recombinant sequences were identical (100 %) to previously described *B. rossi* sequences of black-backed jackals in South Africa, and 99 % similar to *B. rossi* from dogs in South Africa and the Sudan. Although blood specimens from 5 (10 %) of 52 free-ranging African wild dogs (from Kruger National Park, South Africa, reacted with the *B. rossi* probe on RLB hybridisation, the presence of *B. rossi* could not be confirmed by amplification and sequencing, nor by multiplex, real-time PCR. Although African wild dogs can be infected with *B. rossi* without showing clinical signs, our findings suggest that they are apparently not important reservoir hosts of *B. rossi*.

Pathogenesis of ticks and tick-borne pathogens

Room Internacional II, Meliá Internacional Varadero

P44

Unveiling Pathogen Interactions in *Hyalomma* Ticks: Insights from Central Algerian Steppe Regions

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Abstract

Ticks play a crucial role in spreading numerous pathogens affecting both humans and animals. Recently, research has shifted towards exploring pathogen-pathogen interactions using network analysis coupled with high-throughput pathogen detection data, marking a new trend in the field. This study offers the first insights from Algeria into detecting various pathogens and their interactions within *Hyalomma* ticks collected from cattle in the central Algerian steppe regions across three time-points within a single year. A total of 166 tick pools (94 male pools and 72 female pools) were analyzed using microfluidic-based high-throughput real-time PCR to detect 47 different pathogens. Male ticks exhibited an infection rate of 57.45%, primarily dominated by *Rickettsia* spp. (47.62%), with notable occurrences of *Rickettsia africae*. Other significant pathogens included *Rickettsia slovaca* (23.81%), *Rickettsia conorii* (9.52%), and Apicomplexa spp. (7.94%), alongside sporadic instances of various other pathogens. Female ticks demonstrated a slightly higher infection rate of 62.5%, with prevalent pathogens being *Rickettsia slovaca*, Apicomplexa spp., *Borrelia afzelii*, and *Rickettsia* spp., accompanied by additional pathogens detected at varying frequencies. These findings underscore the complexity of pathogen transmission dynamics within tick populations and emphasize the importance of considering both temporal and gender-specific variations in pathogen prevalence. Furthermore, the identification of pathogen-pathogen interactions provides valuable insights into the potential for synergistic or antagonistic effects among co-infecting pathogens, which could have significant implications for disease transmission and control strategies. This study highlights the utility of integrated approaches combining advanced molecular techniques with ecological and epidemiological insights to unravel the intricate interplay between tick-borne pathogens.

KEYWORDS

Ticks, *Hyalomma*, cattle, pathogens interactions, network analysis

Pathogenesis of ticks and tick-borne pathogens

Room Internacional II, Meliá Internacional Varadero

P45

Comparative analysis of bovine blood microbiome in two provinces of South Africa using 16S rRNA PacBio approach

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Ticks are obligate ectoparasites and are good indicators of disease distribution and epidemiology. Recent years has seen a growing concern about the emerging and re-emerging of economically important tick-borne pathogens of livestock and humans worldwide. The overall objective of the study was to give an insight into current tick distribution and associated pathogens that may pose a threat to cattle and potentially humans in the sampled study sites. About 150 cattle were randomly selected from three study sites, Harrismith and Phuthaditjhaba in Free State Province and Bergville in KwaZulu Natal province. Blood samples were collected from the cattle and DNA was subjected to the 16S rRNA gene microbiome sequencing using the circular consensus sequencing approach on the PacBio sequencing platform. Ticks were also collected from the various predilection sites of the sampled animals. A total of eight tick were identified and *Rhipicephalus evertsi evertsi* (79.4%) was the most abundant tick species, followed by *R. appendiculatus* (11.7%), *R. afranicus* (2.6%), *R. simus* (2.6%), *H. rufipes* (1.2%), *R. decoloratus* (0.9%), *H. truncatum* (0.7%) and *R. microplus* (0.7%). The microbiome sequence analysis revealed up to 16 phyla and 30 classes in the three study sites. Proteobacteria was the most dominant bacterial phyla with a relative abundance of 67.2% (Bergville), 73.8% (Harrismith) and 84.8% (Phuthaditjhaba), followed by Firmicutes at 9.6% (Phuthaditjhaba), 18.9% (Bergville) and 19.6% (Harrismith). The Chao 1 index estimator revealed significant differences in the α -diversity of microbial communities between three study sites. This study expands the knowledge on tick fauna and microbial communities in the three study sites.



CUBA - 2024

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P46

Identification of peptides containing B-cell epitopes of the VDAC and RI-86 proteins of *Rhipicephalus linnaei* and assessment of their immunogenicity.

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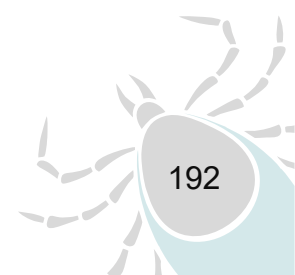
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The tick *Rhipicephalus linnaei*, formerly called *Rhipicephalus sanguineus* s.l. is distributed worldwide due to its ability to adapt to urban and non-urban environments. *R. linnaei* is a vector of multiple pathogens such as *Babesia vogeli*, *Hepatozoon canis*, *Anaplasma platys*, and *Rickettsia rickettsii*, among others. It infests multiple hosts, mainly dogs and occasionally human beings, therefore the development of immunological control methods against this tick species is relevant to human and animal health. In this study, we identified peptides containing B-cell epitopes in the voltage-dependent anion channel (RI-VDAC) and a homologue of Bm-86 (RI-86), from *Rhipicephalus linnaei* followed by an evaluation of their immunogenicity. For this, 3 conserved peptides in the RI-VDAC (RI-VDAC 1, 2 and 3) and RI-86 (RI-86 1, 2 and 3) antigens were selected by bioinformatics analysis tools (ABCpred, BcePred and BepiPred). The peptides were commercially synthesized, and used in an immunization assay wherein vaccine doses were prepared for each peptide at a concentration of 100 µg in a final volume of 1 ml, combined with a commercial adjuvant (Montanide[®] ISA 71 vg). Then, 2 8-weeks old, New Zealand rabbits were immunized subcutaneously with each peptide four times, every three weeks. The generation of specific antibodies against each peptide was determined by indirect ELISA, and the capacity of these antibodies to recognize the native protein obtained from semi-engorged female tick midguts was determined by western blotting and, by immunohistochemistry, using paraffin-embedded tick tissue sections. The results showed that RI-VDAC peptide 2 and RI-86 peptides 1 and 2 are immunogenic. RI-VDAC peptides 1 and 3 as well as RI-86 peptide 3 did not generate a humoral immune response in vaccinated rabbits. In conclusion, the RI-VDAC and RI-86 proteins of *R. linnaei* contain immunogenic peptides that can be included in a multi-antigen and multi-epitope vaccine against this tick species.



**Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines**
Room Guamá, Meliá Internacional Varadero

P47

Identification of biomarkers after *Ixodes ricinus* tick bite exposure as a diagnostic and surveillance tool

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The escalating spread and density of tick populations underscore the urgent need for enhanced surveillance and risk assessment strategies for tick-borne diseases (TBD). Biomarkers derived from the antibody response to tick saliva can be used to facilitate the surveillance of vector establishment in novel regions, assess anti-vector interventions and diagnose TBD through documentation of antecedent tick bites in suspected cases. Here, we derived short peptides from previously identified immunogenic proteins—namely IrCRT, IrSPI, and IrLIP—through bioinformatic predictive analysis using the Immune Epitope Database. ELISAs performed with experimentally controlled sheep sera infested with *Ixodes ricinus* were used to assess the level of antibody response of IgM and IgG to the peptides derived from these three proteins. Next, we tested the candidates on sera derived from both field and clinical isolates of tick-exposed individuals. For all sera, we obtained different IgM and IgG responses with varying degrees of immunogenicity detected per peptide. Further, through use of an exploratory microarray assay (PepperPrint™), we selected new peptides based on their ability to be recognized using serum from experimentally infested sheep. Amongst the top randomly generated peptides, we found a more specific immunogenic response against the IgG antibodies when compared to IgM. Selected candidates were further tested against experimentally-controlled infested sheep sera, as well as field and clinical isolates. Finally, candidates were cross-validated against mosquito-exposed sera to ensure vector specificity. This study offers the potential for developing new effective strategies for the surveillance and diagnosis of tick-related risks as well as the control and prevention of TBD.

**Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines**
Room Guamá, Meliá Internacional Varadero

P48

In silico methodologies combined in vivo assessment of immunogenicity to uncover
Rhipicephalus bursa- Babesia ovis interactions.

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Babesiosis is a disease caused by the Babesia parasites transmitted by ticks and affecting both animals and humans. Babesia bovis and B. bigemina are known to significantly affect meat production while, for example, B. ovis mainly affects small ruminants and has a high prevalence in Mediterranean countries, including Portugal. We have been developing various approaches to study the interactions between this species and its preferred vector, the tick Rhipicephalus bursa.

As published, transcriptomes of R. bursa was produced and analyzed considering both infection and feeding conditions and, transcripts showing clearly differentiated expression in these situations, were selected. A similar approach was performed for the proteome. Gene silencing, mediated by RNA interference, confirmed the function and involvement of genes and proteins in the infection and feeding processes.

Based on the results obtained up to this point, we conducted a study focused on evaluating the immunological characteristics of the targets we selected.

Using a reverse genetics immunoinformatic approach, we identified nine top candidates, comprising one transmembrane-related protein and eight secreted proteins. These candidates exhibited greater predicted antigenicity compared to the Bm86 antigen, while also lacking homology to mammalian hosts and featuring exposed regions. Only four candidates were functionally annotated and chosen for additional in silico analysis. Regions containing overlapping coincident epitope groups (CEGs) were analysed to identify peptides derived from the three R. bursa proteins. In silico findings suggested that the designed epitopes could elicit a protective and enduring immune response against these tick proteins, underscoring their potential as anti-tick vaccines.

The immunogenicity of three peptides was assessed in a preliminary immunization trial using Mus musculus. After immunizations the immune response was assessed by ELISA. By integrating in silico methodologies with in vivo assessment of immunogenicity, will allow to point vaccine candidates before conducting costly studies on the definitive ovine host animals.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P49

Production of monoclonal antibodies against *Babesia ovis* relevant proteins

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The protozoan *Babesia ovis* causes ovine babesiosis, responsible for high mortality and morbidity in infected animals, contributing to significant economic losses. Being mainly transmitted by the tick *Rhipicephalus bursa*, its distribution overlaps with that of the vector found in the Mediterranean region. Since there is no active surveillance of this disease, its prevalence is certainly underestimated. Control of this and other babesioses can be directed either at the etiological agent or at the vector tick that transmits it, but the existing options are limited, ranging from the administration of anti-*Babesia* drugs or attenuated vaccines to the application of acaricides. These limitations support not only the need to strengthen epidemiological surveillance but also progress in the discovery of new anti-*Babesia* drugs.

The current study focuses on the development of a serological diagnostic using poly/monoclonal antibody (mAbs) production technology. Both the apical membrane antigen-1 (AMA-1), which is exclusive to Apicomplexa, and the *B. ovis* surface protein D (BoSPD), thought to be specific for this species.

Briefly, the two recombinant proteins were expressed in a *E. coli* system and different expression strategies were assayed for optimization. Further purification was achieved by using HisTrap affinity chromatography columns. Western blotting allowed confirming the presence of the expressed recombinant protein and its degree of purity. Purified antigens (20ug) containing adjuvant (1:1) were immunized in *Mus musculus* BALB/C mice for polyclonal and monoclonal production. Cell fusion and hybridoma technology will allow the obtaining of cells producing specific antibodies. mAbs are well-established tools for proteomic research and have enormous application in various areas, mainly in the diagnosis of veterinary and/or human diseases. The produced antibodies will be initially used for recognizing the *B. ovis* parasite in culture.

**Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines**
Room Guamá, Meliá Internacional Varadero

P50

Dissecting Hazara virus-tick dynamics: the role of viral-derived DNA forms in *Hyalomma marginatum*

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The Hazara virus (HAZV) is as an important model in the study of viral-tick interactions due to its genetic relationship with Crimean-Congo hemorrhagic fever virus (CCHFV). *Hyalomma* spp. ticks, which preferentially feed on humans, act as both vectors and reservoirs, facilitating the transmission and maintenance of CCHFV in nature. CCHFV is expanding its distribution to areas where it was not found before, representing a growing risk to public health

A deeper knowledge on the molecular interactions between CCHFV and ticks is scarce but essential to develop safe and effective control measures such as vaccines, as done for other tick transmitted virus.

To address this gap, our research began with the establishment of a HAZV culture in mammalian cell lines, allowing us to determine optimal viral infection parameters and produce a reliable viral stock.

Subsequently, we cultivated colonies of tick cell lines, including HLE/LULS42, HAE/CTVM9, and ISE6, to assess their susceptibility to HAZV using RT-qPCR. Ticks were then infected via the immersion method with infection efficacy quantified through the measurement of HAZV RNA levels.

Our study also focuses on the production of HAZV-derived DNA forms within these artificially infected ticks, utilizing both conventional and quantitative PCR techniques. Furthermore, we are longitudinally assessing the presence of HAZV RNA and vDNA forms in key tick organs—namely the midgut, ovary, and salivary glands—via RT-qPCR. This comprehensive approach will enhance our understanding of virus-tick interactions and potentially inform future research and development of targeted control strategies.

This work could yield important insights into the viral-tick interface, particularly concerning the unexplored aspect of viral-derived DNA (vDNA) in RNA virus infections.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P51

**Vaccinomics-based selection and validation of protective salivary antigens from
Ornithodoros moubata.**

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Ornithodoros moubata is the primary vector of African swine fever (ASF) and Tick-borne human relapsing fever (TBRF) in Africa. Developing an effective vaccine against this argasid will facilitate disease control. To identify potential vaccine targets, the recently obtained *O. moubata* sialome was scrutinised using a vaccinomics approach, and a set of salivary secreted proteins predicted to be antigenic and involved in the regulation of blood feeding and host defences were selected.

The aim of this work was to analyse the protective potential of eight of these candidates, namely, Complement inhibitor (OmCI), Hypothetical protein 275 (OmH275), Peroxiredoxin (OmPXR), Cyclophilin (OmCPH), Calreticulin (OmCLR), Neprylisin (OmNEP), Superoxide dismutase (OmSOD) and Peroxinectin (OmPXN).

The candidates were produced as recombinants or synthetic peptides (OmPXN), formulated with Montanide, and administered individually to different groups of rabbits. Adult and nymphal-3 specimens of *O. moubata* and *Ornithodoros erraticus* (the Mediterranean vector of ASF and HRF) were fed on the vaccinated rabbits, and the tick feeding performance, survival, and reproduction rates were evaluated.

OmH275, OmPXR, OmCPH, and OmCLR provided 20% - 32% protective efficacies against *O. moubata* and/or *O. erraticus*, whereas OmCI, OmNEP, and OmSOD provided only 2% - 17% protection against either one or both species, and OmPXN did not protect at all (0%). Accordingly, OmH275, OmPXR, OmCPH, and OmCLR were considered useful candidates to be combined in cocktail vaccine development, searching for cumulative or synergistic anti-tick effects. OmCI, OmNEP, OmSOD and OmPXN were considered little or not suitable for use in tick vaccines.

These results proved the usefulness of this vaccinomics pipeline and validated four of the eight selected candidates (50%) as protective antigens for the development of anti-*Ornithodoros* vaccines.

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CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P52

Microfluidic PCR and network analysis reveals complex tick-borne pathogen interactions in the tropics

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Abstract

Ixodid ticks, particularly *Rhipicephalus sanguineus* s.l., are important vectors of various disease causing agents in dogs and humans in Cuba. However, our understading of interactions among tick-borne pathogens (TBPs) in infected dogs or the vector *R. sanguineus* s.l. remains limited. This study integrates microfluidic-based high-throughput real-time PCR data, Yule's Q statistic, and network analysis to elucidate pathogen-pathogen interactions in dogs and ticks in tropical western Cuba. A cross-sectional study involving 46 client-owned dogs was conducted. Blood samples were collected from these dogs, and ticks infesting the same dogs were morphologically and molecularly identified. Nucleic acids were extracted from both canine blood and tick samples. Microfluidic-based high-throughput real-time PCR was employed to detect 25 bacterial species, 10 parasite species, 6 bacterial genera, and 4 parasite taxa, as well as to confirm the identity of the collected ticks. Validation was performed through end-point PCR assays and DNA sequencing analysis. Yule's Q statistic and network analysis were used to analyse the associations between different TBP species based on binary presence-absence data. The study revealed a high prevalence of TBPs in both dogs and *R. sanguineus* s.l., the only tick species found on the dogs. *Hepatozoon canis* and *Ehrlichia canis* were among the most common pathogens detected. Co-infections were observed, notably between *E. canis* and *H. canis*. Significant correlations were found between the presence of *Anaplasma platys* and *H. canis* in both dogs and ticks. A complex co-occurrence network among haemoparasite species was identified, highlighting potential facilitative and inhibitory roles. Notably, *H. canis* was found as a highly interconnected node, exhibiting significant positive associations with various taxa, including *A. platys*, and *E. canis*, suggesting facilitative interactions among these pathogens. Phylogenetic analysis showed genetic diversity in the detected TBPs. Overall, this research enhances our understanding of TBPs in Cuba, providing insights into their prevalence, associations, and genetic diversity, with implications for disease surveillance and management.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P53

Mediterranean spotted fever on the Crimean Peninsula

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The need to study rickettsioses of the tick-borne spotted fever group explained by the high incidence in the world. On the Crimea, the rickettsioses includes Mediterranean spotted fever, *Rickettsia conorii*, with heterogeneous clinical symptoms. It has been registered since 1936 and the incidence is sporadic and in some years' outbreaks. The incidence in recent years is presented in intensive rates 0.99-2.28 ‰. On the Crimea, dog ticks *Rhipicephalus sanguineus* are almost ubiquitous. Dogs are the main hosts of ticks. At severe infestation of *Rhipicephalus sanguineus* they can attack large and small livestock and cats. Over the past few years, we have been collecting ticks in areas where diseases have been reported. Collections were carried out in nature "for the flag", ticks were examined and removed from animals (dogs, cows, sheep). 3131 specimens of ixodid ticks of 9 species were collected: *Dermacentor marginatus*, *Haemaphysalis punctata*, *Hyalomma marginatum*, *Rhipicephalus sanguineus*, *Ixodes ricinus*, *Rhipicephalus bursa*. Using method of PCR-real time, tick samples were examined for the presence of genetic markers of pathogens of tick-borne rickettsiosis and Mediterranean spotted fever, in particular. The results of PCR with "RealBest DNA Rickettsia species" are as follows: a DNA marker of rickettsia, a region of the citrate synthase gene (*gltA*) was identified; positive samples of rickettsia DNA were selected for sequencing of their sequences (*gltA*, *ompA*, *ompB*, *sca4*); the obtained sequences were compared with rickettsia DNA sequences presented in the NCBI database and the type of rickettsia was determined. As a result of sequencing the circulation of eight species of rickettsia on the Crimean peninsula was established: *R. conorii*, *R. slovaca*, *R. aeschlimannii*, *R. mongolotimonae*, *R. massiliae*, *R. monacensis*, *R. raoultii*, *R. helvetica*, of which - *R. slovaca*, *R. mongolotimonae*, *R. massiliae* and *R. helvetica* were discovered for the first time on the Crimean Peninsula.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P54

PCR targeting the large subunit of phage terminase – a suitable tool for *Borrelia* diagnostic?

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In March 2021 a paper was published suggesting that a real-time PCR targeting the multiple copies of the large subunit of phage terminase (*terL*) located on plasmids cp32 in *Borrelia* improved detection of spirochetes in blood (1). Though an interesting idea, a critical appraisal revealed serious methodological flaws (2).

We have established this real-time PCR in-house and used it to re-analyse serum (n=5) and tissue (n=14) samples, all from patients diagnosed with Lyme borreliosis according to established criteria and with an active *Borrelia burgdorferi* infection. Furthermore, we analysed DNA from *in vitro* cultures of *Borrelia* species (n=36) and a DNA panel consisting of serial dilutions of *Borrelia* DNA from 16 species (n=94).

Five serum samples tested so far from patients with active disease remained negative in *terL* PCR. Out of 14 tissue samples that were positive in control PCRs (*flaB*, 5S-23S IGS), seven remained negative (no Ct value) using the Phage terminase PCR. Species determination revealed that four of the seven negative samples belonged to *B.afzelii*, and three to *B.garinii/B.bavariensis*. Notably, *B.afzelii* and *B.garinii/B. bavariensis* are the main causative agents of Lyme borreliosis in Europe. DNA analyses using cultured isolates of various species of *Borrelia* including *B. afzelii*, *B. burgdorferi sensu stricto*, *B. bavariensis*, *B.garinii*, *B.spielmanii* and *B.valaisiana* showed that some isolates of *B.garinii* and *B.afzelii* remained negative in *terL* PCR, even when 10⁴ DNA equivalents/PCR reaction were used and control PCRs were positive. We have shown using whole genome sequences that these species contained cp32 plasmids. The data suggest that the PCR targeting the *terL* locus in its current form is unsuitable as diagnostic tool for *Borrelia* in Europe, especially from serum samples.

Reference:

- (1) Shan et al. (2021) Front. Microbiol. 12:651217.
- (2) van de Schoor et al. (2021) Front Microbiol. 13: 802131.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P55

Real Time PCR for Detection of *Rickettsia* sp. in *Rhipicephalus sanguineus* collected from areas of extreme poverty in Peru.

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The geographical and epidemiological characteristics of Peru contribute to the circulation of several blood-sucking arthropods involved in the transmission of pathogens from human or animal reservoirs to susceptible hosts. These include *Rhipicephalus* and *Amblyoma*.

Rickettsiosis are one of the many neglected vector-borne bacterial aetiologies. Therefore, the aim of this project is the development of a real-time PCR method for simultaneous confirmatory molecular diagnosis of rickettsiosis in vectors.

Methodology: Unique and specific gene regions of the complete genomes of the bacterial species associated with the Rickettsiosis were identified by bioinformatic analysis. Genomic scans were performed with the BLAST+ programme, primers and probes were designed with the PRIMER5 programme, their quality was analysed with the OligoAnalyzer; after the synthesis of the designed oligonucleotides and their labelling with different fluorophores, the corresponding molecular assays were performed and then the parameters of the developed method (test standardisation, detection limit, specificity, sensitivity) were evaluated.

Results: we standardized a high-throughput, rapid and inexpensive real-time PCR for the diagnostic scheme of vector-borne bacterial diseases and for the molecular surveillance of neglected bacterial metaxenic diseases of unknown prevalence in different regions of Peru, such as rickettsiosis, which could be related to febrile diseases of unknown origin reported in different localities of Peru. Also, 120 samples of *Rhipicephalus sanguineus* were evaluated, obtained eight positive samples for *Rickettsia* sp, six from the northern regions (for Piura and two Tumbes) and two a southern regions (Tacna). This results could mean that transmission of *Rickettsia* is likely in these areas.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P56

Seroprevalence of tick-borne encephalitis in small ruminants and dogs in the Czech Republic and experimental veterinary vaccine development

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Tick-borne encephalitis virus (TBEV) causes severe neuroinfections in humans in Europe and Asia. Humans are typically infected after a tick bite; however, alimentary infection can occur after consumption of unpasteurized dairy products. Milk from ruminants can be a source of alimentary TBE infections. In addition, sheep and goats are considered suitable sentinels for surveillance of TBEV-associated risks in endemic areas. Performed serological survey showed significantly higher seropositivity among sheep (32.5%) than goats (19.7%). The present results indicate that sheep and goats have a relatively high rate of exposure to TBEV-infected ticks in the Czech Republic.

Also dogs are frequently infected with TBEV (rarely also with clinical manifestation) and can be used as sentinel animals. Therefore, we conducted a serosurvey to assess TBEV exposure in dogs in the Czech Republic. TBEV-specific antibodies were detected in 13% dogs, which confirmed a high, but clinically inappreciable TBEV exposure rate in the endemic area. Two dogs with fatal acute TBE were also described during the study.

We prepared experimental veterinary TBE vaccines for i) immunization of ruminants to prevent alimentary milk-borne TBEV infections in humans and for ii) protection of dogs from severe forms of TBE. Vaccines were developed based on inactivated whole virus antigen. The safety and immunogenicity of the vaccines were evaluated in mice (model organism) and in sheep and dogs (target organisms). Vaccines were well-tolerated while eliciting the production of high levels of virus-neutralizing antibodies. Vaccination provided full protection against lethal TBE in mice. Immunisation prevented development of viremia in dogs and prevented presence of TBEV in milk of lactating ewes after experimental infection. Therefore, the developed vaccine candidates are promising to protect dogs from severe TBEV infections, and prevent alimentary milk-borne TBEV from vaccinated ruminants.

This study was supported by the Ministry of Health of the Czech Republic (project NU21-05-00143).

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P57

High seroprevalence of *Bartonella henselae* in healthy blood donors in the Czech Republic

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The genus *Bartonella* is a rapidly spreading group of ubiquitous bacteria that are mainly found in various animal species, but some of which can also be transmitted to humans. Three species, *Bartonella henselae*, *B. bacilliformis* and *B. quintana*, are responsible for the majority of human cases. Bartonellosis is an infectious disease with clinical manifestations ranging from mild symptoms to severe, life-threatening complications, especially in immunocompromised patients. The main reservoir is the domestic cat. *B. henselae* is transmitted to humans from infected cat flea faeces through bites or scratches. Although the cat flea is the main vector, ticks are also considered potential vectors for *Bartonella* transmission.

Due to the lack of information on the occurrence of bartonellosis in the human population in the Czech Republic, the aim of our study was to determine the seroprevalence against *B. henselae* in healthy blood donors in our country and to compare it with the number of acute illnesses caused by *B. henselae*.

The serum samples of 677 healthy blood donors from 2023 were analysed for *B. henselae* IgG antibodies using indirect immunofluorescence assays. In 2023, we collected 368 samples from patients with acute symptoms such as lymphadenopathy or other symptoms of bartonellosis such as fever, weakness and headache.

A high prevalence of 20.4% of IgG antibodies against *B. henselae* in the base titer was found and a high titer of antibodies was found in 2.7% of the donors. In symptomatic patients with lymphadenopathy or other symptoms, the seroprevalence was 51.6%, while a high titer indicating acute disease was found in 9.8%.

The high seropositivity rate for *B. henselae* in healthy blood donors is consistent with our previous findings in a group of patients with other tick-borne diseases. We found significant differences in seropositivity of IgG antibody titers between symptomatic patients and healthy controls.

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Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P58

Genome-wide analysis of *Theileria parva* proteases for identification of potential drug targets

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Theileria parva is an intracellular protozoan parasite that causes theileriosis, a fatal lymphoproliferative disease that has a major economic impact on livestock production in East, Central and Southern Africa. Theileriosis is treated with buparvaquone, but this drug does not eliminate the infection. As a result, treated cattle become carriers and a source of future *T. parva* infection. In addition, resistance to buparvaquone has been reported in the related *Theileria annulata*. Therefore, there is a need to identify alternative drug targets, preferably those that will eliminate the parasite to avoid the carrier state. The survival of organisms depend on the activation or deactivation of their proteins by proteolysis, a process facilitated by proteases. Proteases and their inhibitors are therefore considered to be virulence factors and regulators of disease pathogenesis. These molecules are essential in the life cycle of protozoan parasites, where they are reported to be involved in important processes such as protein homeostasis, host cell invasion, cell signalling, inflammation, and immunomodulation of host responses. Accordingly, they are considered to be good targets for the design of anti-parasitic drugs. An *in silico* approach was employed to identify essential proteases for consideration as anti-*T. parva* drug targets. Analysis of the *T. parva* proteome using a combination of bioinformatic tools resulted in the identification of 115 proteases. Sixty-five of these had homologs in the bovine proteome and were excluded from further analysis. Of the remaining 50 proteases, 19 were identified as transmembrane proteins, of which 12 were predicted to be involved in cell invasion, metabolic pathways, immune processes and reproduction. These proteins are recommended for further investigation as targets for drug therapy against *T. parva*.



CUBA - 2024

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P59

**MOLECULAR DETECTION AND EPIDEMIOLOGICAL ANALYSIS OF *Anaplasma marginale*
IN *Rhipicephalus microplus*, *Stomoxys calcitrans* AND *Haematobia irritans***

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Bovine anaplasmosis is a disease caused by the intracellular bacterium *Anaplasma marginale* (Rickettsiales: Anaplasmataceae). It is widespread and can cause significant economic losses. This study investigates the presence of *A. marginale* DNA in the tick *Rhipicephalus* (*Boophilus*) *microplus* and the flies *Stomoxys calcitrans* and *Haematobia irritans* from dairy farms in Seropédica and Paracambi, State of Rio de Janeiro. In the laboratory, the arthropods were dissected and the salivary glands of the ticks and the proboscis of the flies were isolated to extract their DNA using the phenol-chloroform method. They were then analyzed by nested PCR targeting the *msp5* gene. Positive results were obtained for 73% (n=29/40) of *S. calcitrans*, 90% (n=26/29) of *H. irritans* and 45% (n=43/96) of *R. microplus*, which were sequenced by the Sanger method for confirmation, achieving 98.11% to 100% similarity with other *A. marginale* sequences deposited in GenBank. Pairwise comparison showed 97.81% to 100% of similarity between the sequences from this study. A multivariate statistical analysis was then performed to predict the factors associated with *A. marginale* infection in *R. microplus* using multiple logistic regression analysis. The presence of flies over the animals was considered as a risk factor, with infested animals being 3 times more likely to have their ticks infected with *A. marginale* (p-value= 3.26; OR:3.26; IC:1.17-9.08). This study is preliminary and highlights the possibility of these hematophagous dipterans being the main vectors of *A. marginale* in the epidemiological chain of the disease. It also highlights the importance of experimental research and the implementation of new strategies to prevent all possible vectors of this pathogen that affects so many cattle around the world.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P60

Conventional multiplex PCR for detection of tick-borne pathogens: proof of concept and utility in biological samples

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Ticks, as hematophagous acarids, are known vectors of various pathogens of veterinary and human health concern, including *Borrelia* spp., *Babesia* spp., and *Anaplasma phagocytophilum* in Europe. Detection of those pathogens in ticks is necessary to evaluate prevalence and then making recommendations for public health. Conventional PCR is still commonly used in labs despite being quite time-consuming and expensive. Multiplex PCR, by allowing the detection of multiple pathogens within the same PCR, reduces both the financial cost and the time needed to screen biological samples for multiple tick-borne pathogens.

Here, by using different multiplex PCR, we combined the detection of 5 pathogens of medical interest. We used previously described primers known for their efficiency in detection of pathogens of the same genus or taxon group. As multiplex were successfully tested with one, two or three pairs of primers, the right multiplex PCR can be chosen regarding the research objective. One multiplex PCR allows the detection of *A. phagocytophilum*, *Borrelia burgdorferi sensu lato* and parasites of both *Babesia* and *Theileria* genera. Bacteria of the genus *Bartonella* and *Francisella tularensis* subspecies can also be detected together, with *A. phagocytophilum*.

Both multiplex PCR were validated by plasmid control and will be implemented on biological samples coming from Ile de France region of France. Sensitivity and specificity will be also assessed. These multiplex PCR were designed to be used routinely for the detection of pathogens in ticks from field collections and thus offer a step towards improved cost- and time-effectiveness for surveillance of tick-borne pathogens.

Keyword: tick-borne pathogens, PCR, multiplex.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P61

Generation of a recombinant single variable domain (VHH) antibody from the *Lama glama* model against a *Babesia bovis* antigen.

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The immune system of camelids expresses antibodies that lack light chains, called heavy chain antibodies (HCAB). The antigen binding site of HCABs is known as the variable heavy chain (VHH) domain or nanobody. VHHs are the smallest known antibodies and are characterized by high stability, solubility, specificity, low immunogenicity, good tissue penetration, easy production in microorganisms, and low-cost manufacturing. Therefore, VHH fragments are considered as an alternative for different therapeutic, diagnosis and research applications. In this study, a methodology for the generation of a recombinant VHH antibody was applied, using the recombinant model of the Translationally Controlled Tumor Protein (rBboTCTP) of *Babesia bovis*. To obtain the VHH fragments, a llama (*Lama glama*) was immunized with rBboTCTP protein at a concentration of 100 µg in a final volume of 1 ml, combined with a commercial adjuvant (Montanide™ ISA 201 VG). The generation of specific VHH antibodies to the protein was determined by indirect ELISA. Individual isolation of VHH-secreting plasma cells specific against rBboTCTP protein was performed by micromanipulation, using fluorophore labeling. The cDNA of each cell was obtained using a commercial kit (SuperScript™ IV Single Cell/Low Input cDNA PreAmp Kit) and the sequence of each fragment was amplified by PCR. As a result of this reaction, the sequence of 3 fragments from individual cells were obtained, cloned into a commercial vector and sequenced using SANGER automated methodology. The sequences obtained were analyzed and compared with the sequences deposited in public databases, to determine by molecular docking their interaction with the rBboTCTP protein. With these assays, we aim to demonstrate that the complete sequence of a VHH fragment against TCTP protein was obtained for its subsequent expression in bacterial models, and with this, to achieve in the future a method to obtain specific antibodies against antigens of parasites of the genus *Babesia*.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P62

Evaluation of integrated One Health economic policies to control Crimean Congo Hemorrhagic Fever

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Background

Crimean Congo Hemorrhagic Fever (CCHF) is a highly fatal neglected tick-borne zoonosis. However, the developing countries are more at risk, particularly the tropical countries are considered as favourable case sites. There is a lack of integrated policies to control CCHF in humans and livestock and habitat for Ixodid (hard) Hyalomma ticks, which act as both reservoir and vector for the CCHF virus.

Methods

An economic analysis was done using a comprehensive disease control policy options model named HandEcon (Human and Animal Disease Economics). A cost-effective analysis (CEA) was performed to evaluate the three alternative control options of CCHF for the duration of ten years with 5% discount rate and to recommend the cost-effective policy option to decision makers. The options included different combinations of integrated surveillance, awareness campaign and vector Control.

Results

Based on the CEA, both the Net cost per human case avoided (CU per case) and Net cost per DALY avoided (CU per DALY) are lowest in the option of Awareness Campaign only. As this disease is asymptomatic in animals, the final incidence in humans will be considered which is also less here. Moreover, the Cumulative discounted control costs (CU) is lowest in 'Awareness only' option.

Conclusion

Economic analysis outcomes were strongly influenced by costs of control options, population sizes in control areas, cost of human treatment due to CCHF and effectiveness of control in reducing CCHF incidence in humans and animals. CCHF is largely undiagnosed due to lack of awareness among health professionals. Therefore, One Health collaboration between animal health and human health on CCHF at all levels is vital to its overall detection, prevention and control.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P63

IRIXIN - a novel contact phase coagulation inhibitor from the tick *Ixodes ricinus*

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Contact factor XII is an interesting target for the development of a next generation of anticoagulants. Inhibition of FXII has emerged as a therapeutic strategy for the safe prevention of thrombosis induced by artificial surface and in patients suffering from hereditary angioedema. Compared to concomitantly used anticoagulants (Thrombin and FX inhibitors), FXII inhibition does not lead to excessive bleeding.

The aim of this study was to investigate the anticoagulant and antithrombotic activity of Irixin, a novel Kunitz-type protein of 171 amino acids that was discovered in the gut of the tick *Ixodes ricinus*. In vitro, we have shown that Irixin prolongs blood clot formation, inhibits thrombin and kallikrein formation, and interacts with activated human contact phase factors FXIIa and FXIa. The interaction with XII and XI makes Irixin an interesting target for the development of thromboembolic drugs with a lower risk of treatment-related bleeding.

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CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P64

**Integrated Approach for Assessing Tick-Borne Pathogens in South African Horses:
Quantitative Detection of *Theileria haneyi* and Molecular Genotyping of *Babesia caballi***

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Haemoprotzoan parasites, *Theileria equi*, *Theileria haneyi* and *Babesia caballi* are causative agents of a disease of equids, known as equine piroplasmiasis. Molecular genotyping of *T. equi* parasites revealed the existence of five *T. equi*-like genotypes, and an association between genotype C and *T. haneyi* was suggested. A nested PCR assay to distinguish between these parasites proved unreliable, but the exclusivity of the *ema-11* gene in *T. haneyi* provided an opportunity to develop a more specific and sensitive qPCR assay for parasite detection. Similarly, variant *B. caballi* genotypes reported globally compromised the diagnostic utility of WOAH-recommended serological assays for declaring horses free of parasites. Given the recent discovery of the spherical body protein 4 (SBP4) gene as a potential antigen for serological detection of *B. caballi*, this study further outlines the development of molecular typing assays based on this gene for the detection and quantification of the three known *B. caballi* parasite genotypes. The *T. haneyi ema-11* (*Thema-11*) qPCR assay was shown to be rapid, specific, and sensitive in detecting *T. haneyi* infections. The diagnostic utility of the *Thema-11*-specific qPCR assay was evaluated together with a *T. equi ema-1*-specific qPCR assay. The results suggest that used in combination, the *Thema-11*-specific qPCR assay and the *T. equi ema-1*-specific qPCR assay could detect and differentiate between *T. haneyi* and *T. equi* infections. Additionally, all three *B. caballi sbp4* qPCR assays had acceptable detection limits and were specific in the detection of the relevant *B. caballi* genotypes from *in vitro* cultured and field samples from South Africa. Despite challenges posed by variant parasite genotypes the development of specific qPCR assays targeting the *ema-11* gene for *T. haneyi* and the SBP4 gene for *B. caballi* offers promising improvements in parasite detection and differentiation, aiding in the management and control of equine piroplasmiasis.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P65

Development of cocktail vaccines against *Ornithodoros argasid* ticks.

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Ornithodoros erraticus and *Ornithodoros moubata* are the primary vectors of Tick-borne human relapsing fever (TBRF) and African swine fever (ASF) in the Mediterranean basin and Africa, respectively. Developing effective anti-*Ornithodoros* vaccines will reduce parasite populations and facilitate disease control.

Candidate vaccine targets have primarily been sought in ticks' salivary glands and midgut because these organs express proteins specifically evolved by ticks to adapt to strict hematophagy. As these proteins play important roles in blood meal acquisition, digestion, and regulation of host defences, they may be promising vaccine targets.

Scrutiny of the sialomes and mialomes of both *Ornithodoros* species through vaccinomics approaches has identified a repertory of salivary and midgut antigens that individually confer around 50% protection against *O. erraticus* and/or 40% against *O. moubata*.

Combining these protective salivary and midgut antigens is expected to broaden the protection range and enhance the efficacy of tick vaccines by simultaneously targeting several biological processes in different organs.

The aim of this work was to assess the vaccine efficacy of two cocktail vaccines consisting of a combination of protective salivary and midgut antigens from either *O. erraticus* (OEmix) or *O. moubata* (OMmix) in rabbit immunization trials.

OEmix provided 73% protection against *O. erraticus*, confirming the hypothesis of enhanced protective efficacy for this cocktail vaccine. By contrast, OMMix provided 40% protection against *O. moubata*, similar to individual antigens. Possible causes influencing this outcome (e.g., antigenic competition) and ways to improve it are discussed.

OEmix and OMMix also provided 33% cross-protection to the heterologous species, supporting the hypothesis of an enhanced protection range.

These results encourage further investigation aimed at identifying new protective antigens and developing anti-tick cocktail vaccines.

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CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P66

***Ehrlichia* Species in Dromedaries and Ruminants from Somalia: With the First Report of *Ehrlichia minasensis* in Dromedaries, Sheep, and Goats Globally**

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Abstract

Background: Ehrlichioses, caused by *Ehrlichia* species, are tick-borne diseases affecting animals and humans globally. Heartwater, caused by *E. ruminantium*, is economically significant in African ruminants. *Ehrlichia minasensis*, a newly identified agent, may affect cattle. In Somalia, the tick-borne diseases (TBDs) status is unknown. This study explores *Ehrlichia* spp. in 530 animals (155 dromedary, 199 goat, 131 cattle, and 45 sheep) from Benadir and Lower Shabelle regions in Somalia.

Material and Methods: Ruminant and dromedary blood DNA samples were subjected to PCR assays targeting the *dsb* gene of *Ehrlichia* spp. and *PCS20* gene of *E. ruminantium*. PCR-positive samples were submitted to PCR assays targeting *sodB* gene of *Ehrlichia* spp. and *map1* gene of *E. ruminantium*, followed by Sanger sequencing, BLASTn and phylogenetic analyses.

Results: Overall, 140/530 (26.4%) animals tested positive for *Ehrlichia* spp. based on the *dsb* gene, with high prevalence in dromedaries (54.8%), cattle (29.8%), goats (7.0%), and sheep (4.4%). Among *dsb* PCR-positive samples, 30/39 (76.9%) cattle tested positive for the *sodB* gene, while samples from dromedaries, goats, and sheep were *sodB* gene-negative. Eighteen out of 131 cattle (13.7%) tested positive for *E. ruminantium* based on the *pCS20* gene. However, the *E. ruminantium map1* gene assay returned negative. Significant associations were found between *E. ruminantium* positivity, cattle body condition, and tick presence ($p < 0.001$) in cattle. The detected *dsb* gene sequences showed 98.5-100% identity with *E. minasensis*, and the *sodB* gene displayed 83-85% identity with *Ehrlichia* spp. strain H7 and 83.4% identity with *E. ruminantium*.

Conclusion: The present study showed, for the first time, molecular evidence of *E. minasensis* in dromedary and ruminants from Somalia, as well as globally in dromedaries, sheep, and goats. Furthermore, *E. ruminantium* is detected in Somali cattle, emphasizing the need for further research to understand the economic and public health implications.

Keywords: Tick-borne diseases, Ehrlichiosis, *Ehrlichia minasensis*, *Ehrlichia ruminantium*, Heartwater, Sub-Saharan Africa.



CUBA - 2024

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P67

***In vitro* evaluation of OleoVET and formulations action on reproductive indicators of teleogins
(Rhipicephalus microplus).**

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Abstract

Rhipicephalus microplus is considered the most important tick of cattle worldwide and a vector of numerous hemoparasitic diseases. Ozonated sunflower oil, called OleoVET for veterinary use, showed a larvicidal action against cultures of *R. sanguineus*. Therefore, the objective of this study is to evaluate the influence of OleoVET and its formulations on reproductive indicators of replete ticks (*Rhipicephalus microplus*). The Teleogins were divided into six groups of 20 each and exposed to following test substances (drinking water, sunflower oil, placebo, OleoVET, 45% and 60% formulations), using the immersion method. The evoo weight, ovoposition inhibition percentage (O.I.), hatching percentage, estimated reproduction (ER) and control percentage, were determined. The results showed a significant reduction in evoo weight of theologines exposed to OleoVET, 45% and 60 % formulations, accompanied by a significant increase in O.I., with respect to the control groups. The OleoVET and formulations 45 and 60 %, significantly reduced hatching percentage and estimated reproduction, compared to controls groups, reaching a reproductive control percentage (57, 55 and 63 %), respectively. It was concluded that OleoVET and the 45 and 60 % formulations were effective in controlling the reproduction of *Rhipicephalus microplus* ticks.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P68

Establishment of a fluorescence-based method for anti-*Babesia ovis* drug screening

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Among the more than 100 species described, *Babesia ovis* is characterized by high pathogenicity in small ruminants, with mortality rates in susceptible hosts ranging from 30 to 50% in field infections, thus causing a severe impact on the animal industry. Currently, babesiosis control measures target either the parasite, using diminazene aceturate or imidocarb dipropionate, or the vector, through the application of chemical acaricides. The establishment of *in vitro* culture of *Babesia* in the erythrocytic phase has been allowing the study of parasite molecules involved in essential infection processes, such as cellular invasion, and assessing the impact of drugs on these processes. Fluorescence-based assays using SYBR Green I (SG I) have been established for the screening of drugs against the *in vitro* growth of other piroplasmida, including *B. bovis*, *B. bigemina*, *Theileria equi* and *B. divergens*. Such implementation has allowed the rapid screening of hundreds of compounds in the last years. Therefore, the present study aims to establish a fluorescence-based protocol to evaluate *B. ovis* growth inhibition and after test a panel of compounds predicted to inhibit parasite cysteine proteases. *B. ovis in vitro* cultures were maintained in 10% (vol/vol) defibrinated lamb erythrocytes in a HEPES-buffered Medium 199 (1x) containing 20% lamb serum, in a microaerophilic, stationary-phase culture system. The linearity between relative fluorescence units (RFU) and parasitemia was evaluated by following a 1% parasitaemia culture by both light microscopy and fluorescence quantitation after SG I staining during 96h with and without daily replacement of the culture medium. Also 2,5% and 10% hematocrit was tested. The Z' factor was calculated to determine the best conditions for high throughput screening assays. In parallel, based in the literature and previous experiments, Blasticidin and Atovaquone in supra lethal concentrations were evaluated as antiprotozoan agents in light microscopy to be used in posterior assays as controls. Subsequently, the fluorescence method and microscopy were used to determine 50% inhibitory concentration (IC50) of Imidocarb dipropionate and tulathromycin. The optimization of the protocol allowed the screening of compounds with a potential impact in *B. ovis* growth, assays which are currently being performed.



CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines**
Room Guamá, Meliá Internacional Varadero

P69

**Diagnostic difficulties in a clinical case of tuberculous meningo-encephalitis complicated by superinfection
with West Nile virus**

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Climate change induced by global warming is causing many emerging infectious diseases.

Thus, hot periods followed by heavy rains increase the number of infectious vectors (mosquitoes, ticks) and recent studies indicate an increased resistance of these species to pyrethroid insecticides. As a result, cases of acute meningoencephalitis appear more and more frequently, in which differential etiological diagnosis is difficult.

I present to you a clinical case of TBC meningoencephalitis, complicated by a flaccid crural paresis caused by the overlap of a West Nile Virus neuroinfection, in a patient admitted to the Clinical Hospital for Pneumophthisiology and Infectious Diseases in Brasov, Romania.

According to the National Institute of Public Health, in Romania only in 2023, during the surveillance period between June 6 and October 12, 82 cases of West Nile virus infection were reported, of which 75 were confirmed and 7 probable, 10 of those cases resulted in death.

The immunodepression installed following the isolation during the Covid pandemic, the lack of access to a specific treatment, the decrease in the number of seasonal vaccinations, but also of those included in the national programs for the population and for the animals, in addition to the population migration due to the recent wars, creates an increased pressure on the public health services.



CUBA - 2024

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P70

Loop-mediated isothermal amplification (LAMP) assay for rapid visual detection of *Anaplasma marginale* infection in bovines after anti - tick vaccination treatment.

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Abstract

Ticks are recognized as the second most significant vectors of pathogens to both humans and animals, following mosquitoes. Haemoparasitic infections transmitted by ticks result in annual losses amounting to billions of U.S. dollars in the livestock industry, predominantly impacting cattle production in tropical and subtropical regions. Among the diseases most important in this area are Anaplasmosis and Babesiosis. Anaplasmosis in bovines is caused by *Anaplasma marginale*, a bacterium biologically transmitted by *Rhipicephalus* spp. ticks and mechanically transmitted by biting flies, blood-contaminated needles, and farm equipment. Diagnosis of *Anaplasma* spp. infections traditionally relies on serologic tests, though more recently, a variety of molecular diagnostic assays have been developed. Various PCR techniques, including nested PCR, semi-nested PCR, and RT PCR, have been employed for the detection of *Anaplasma* infection in blood and tissue samples, targeting genes such as 16S rRNA, msp4, groEL, ankA, and p44. However, these assays are not practical for large-scale surveillance. Therefore, a cost-effective, simple, rapid, specific, and sensitive method is critical for monitoring hemoparasite infection in cattle. The primary aim of the present study was to develop and evaluate a loop-mediated isothermal amplification (LAMP) assay for the rapid visual detection of *A. marginale* in bovines. A set of six LAMP primers, including two outer primers, two inner primers, and two loop primers, was designed. The same gene target sequence (major surface protein 1α) of *A. marginale* cloned in a T vector was used as a positive control for LAMP assays. To facilitate the use of this diagnostic tool in field conditions, DNA extraction was performed using a simple boiling method. Time and temperature conditions for specific amplification were optimized at 65°C for 60 minutes, with the addition of SYBR Green I, allowing visual detection of positive amplifications. Under these conditions, positive and negative samples were successfully identified directly from cattle blood.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P71

Obtention of recombinant *msp5* antigen to develop an ELISA for *Anaplasma* spp. diagnostic

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ABSTRACT

The genus *Anaplasma* (Rickettsiales: Anaplasmataceae) includes obligate intracellular Gram-negative bacteria primarily transmitted by ticks, encompassing six species: *Anaplasma bovis*, *Anaplasma centrale*, *Anaplasma marginale*, *Anaplasma phagocytophilum*, *Anaplasma platys*, and *Anaplasma ovis*. Notably, *A. phagocytophilum* poses a significant zoonotic threat. These pathogens are responsible for veterinary diseases that impact domestic and wild animals globally, leading to substantial economic losses in the livestock industry and increasing human infection cases. The clinical diagnosis of *Anaplasma* infections is challenging due to nonspecific symptoms that vary with the specific agent, host, immune status, and co-infections. The urgency for precise diagnostic tests is heightened by the economic impact on livestock, the zoonotic potential, and the risk of transfusion-transmitted infections. Six major surface proteins (MSPs) of *Anaplasma* spp., which harbour epitopes B and T, have been characterized and designated MSP1a, MSP1b, MSP2, MSP3, MSP4, and MSP5. Among them, recombinant MSP5 has emerged as a pivotal antigen for the serological diagnosis of *Anaplasma* spp. through enzyme-linked immunosorbent assay (ELISA). This study reports the standardization of an indirect ELISA assay for detecting antibodies against *Anaplasma* spp. using recombinant MSP5. The *Anaplasma msp5* gene was successfully cloned, expressed, and purified in *Escherichia coli*, achieving protein purity exceeding 95%. This purified MSP5 protein was utilized to coat ELISA plates for evaluating bovine sera. The ELISA demonstrated high specificity and sensitivity, effectively distinguishing *Anaplasma*-positive from -negative sera, thereby validating MSP5 as a highly suitable antigen for serological diagnosis. These results highlight the potential of this standardized ELISA to enhance diagnostic accuracy, facilitating early detection, and improving disease management strategies for *Anaplasma* infections.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P72

Amplification of microsatellites of *Rhipicephalus sanguineus* ticks using specific primers from *Rhipicephalus microplus* ticks.

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Ticks are considered the second most important transmitters of diseases in humans after mosquitoes. They affect domestic and wild animals. Immune control is a promising alternative for its control; however, there aren't available 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. *R. sanguineus* tick colony has been established at 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 six loci of them. PCR products for both species were sequenced to corroborate the specificity of amplicons. Effective cross-amplification was 37.5%. The selected microsatellite loci could be used to assessment genetic variation of specimens from "Bejucal 2010" tick colony.

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P73

A NOVEL METHOD FOR DETERMINING THE EFFICACY OF ACARICIDES UNDER FIELD TRIAL CONDITIONS.

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Abstract

In Uganda, there is a regulatory requirement for newly formulated or introduced acaricides to undergo field trials before registration and licensing. We reviewed the various recommended methods for determining the field efficacy of new acaricides by reputable organizations such as FAO, European Medicines Agency, and Medicines Control Authority of Zimbabwe, Uganda's National Drug Authority, WAAVP and methods proposed by other scholars. The recommended methods were found to have some shortcomings affecting the quality of the trial results. Some key shortcomings identified included; failure of the methods to consider the use of a comparable control product, assuming use of a placebo in the control group, failure to meet key principles of randomized clinical trials (RCT) and failure to account for realities in field settings. With all the shortcomings of the recommended methods and gross insufficiency of published literature about acaricide field trials, it was fascinating to advance a novel method for acaricide efficacy determination under field trial conditions. Using this novel method, the efficacy of trial products can be determined for any day of the cycle post acaricide application by determining the relative risk of tick infestation (RRTI) among the animals on farms exposed to the trial product and among animals on farms exposed to control product using the formula below:

$$RRTI = \frac{at(dc + ac)}{ac(dt + at)}$$

The method has also put forward the desired outcome for acaricide application, variable for measurement of outcome, developed data capture form for trial data collection, developed formulae for determining efficacy, stated assumptions of the method, provided guidance on results interpretation and decision making. Lastly, the method meets the principles for RCT and GCP trials thus generating accurate, reliable and credible results. We recommend to researchers and regulatory bodies at local and international levels to embrace and adopt the use of the method to promote accurate, fair and plausible efficacy evaluation of new acaricides in field conditions.

Key Words: Acaricide field trials, relative risk of tick infestation, field trials, methods.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Diagnosis and strategies for control of
ticks and tick-borne pathogens including immunity and vaccines
Room Guamá, Meliá Internacional Varadero

P74

Enhancing East Coast Fever Vaccination: The Potential of TLR Agonists as Adjuvants

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Abstract

East Coast Fever, caused by the protozoan parasite *Theileria parva*, is a tick-borne disease that severely impacts the dairy and beef industries in Eastern, Central, and Southern Africa. The traditional control method, the Infection and Treatment Method (ITM), involves administering a live vaccine concurrently with antibiotics. However, this approach increases vaccination costs and contributes to the risk of antimicrobial resistance, a growing global concern. This study explored an alternative approach by assessing the effectiveness of a Toll-like receptor 7 (TLR7) agonist, which could potentially replace antibiotics in the ITM method. TLRs on antigen-presenting cells (APCs) trigger innate inflammatory responses and stimulate adaptive immunity, enhancing vaccine efficacy. The study involved cattle that were screened for *T. parva* infection using ELISA and PCR, with only uninfected animals included. The vaccine stabilate's potency was confirmed in a preliminary test, after which the main experiment was conducted with two groups of cattle: Group 1 (Test Group) consisted of 15 animals each receiving 1 ml of the vaccine ILRI 4133 at a 1:100 dilution, co-administered with 1 ml of TLR7 agonist at a dose of 1mg. Group 2 (Infectivity Control Group) comprised of three animals, each administered 1ml of the ILRI 4133 vaccine at 1:100 dilution but without the TLR agonist. Over a 21-day period, the cattle underwent parasitological, hematological, and clinical assessments to determine their responses. In Group 1, 40% of the animals were successfully vaccinated with mild to moderate reactions, requiring no ECF treatment, while 60% exhibited severe reactions needing treatment. In Group 2, two of the three animals experienced severe reactions. The findings suggest that TLR7 agonists could enhance vaccine protection against tick-borne diseases like ECF, offering a potential alternative to antibiotics. Future research should investigate using multiple TLR agonists to further improve vaccine efficacy.



CUBA - 2024

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P75

Optimizing tick vaccines with multi-antigenic formulations

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Tick infestations represent a substantial challenge to livestock industries worldwide, necessitating the development of sustainable control methods. Our research team has formulated a multi-antigen vaccine that provides approximately 78% protection against cattle ticks. Despite its efficacy, the vaccine's commercial viability is hindered by the complexity of its production, which requires multiple fermentation processes. To overcome this issue, we are exploring two novel vaccine development strategies: a) chimeric proteins that integrate the full sequences of three distinct protective antigens into a single trivalent construct using protein linkers, and b) single antigens composed of multiple epitopes derived from ten different protective antigens. These innovative approaches aim to streamline production and enhance the feasibility of an anti-tick vaccine. Trivalent chimeric antigen sequences were cloned into plasmids and successfully expressed in an *E. coli* system, demonstrating both purity and scalability for production. For the multi-epitope vaccine strategy, we conducted comprehensive B and T-cell epitope mapping across ten protective antigens. Using Peptide Microarray and Next-Generation Phage Display analyses, we identified linear and conformational B-cell epitopes by employing polyclonal sera from protected bovines. Peptide microarrays revealed six linear B-cell epitopes in 4 out of 9 antigens. Additionally, the Next-Generation Phage Display strategy identified one to three conformational B-cell epitopes per antigen. In silico analyses uncovered 17 CD4 T-cell epitopes across all ten vaccine antigens, corresponding to seven BoLA-II alleles that are prevalent in South American Holstein-Friesian herds. These identified epitopes have been incorporated into the development of epitope-based chimeric antigens, which will be further assessed for their immunogenicity and efficacy. Our results indicate the potential for a commercially viable, easily producible anti-tick vaccine to mitigate *R. microplus* infestations in cattle.

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CUBA - 2024

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P76

Implementation of orthogonal methods to facilitate conjugation site assignment in conjugate vaccines against tick infestation

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Abstract

The conjugation sites are a critical quality attribute of the conjugate vaccines and must be determined. The process of conjugation site assignment is complex, time consuming, and it requires the analysis of hundreds of thousands of MS/MS spectra. In the particular case of a veterinary conjugate vaccine, which does not use cleavable cross linkers, but instead uses low cost cross linkers, assigning conjugation sites reliably is a challenge. Because of this, the use of orthogonal methods that increase confidence in conjugation site assignment is necessary. Aiming to this goal, we implemented new orthogonal methods in the Xion C software developed in our laboratory. 1) extracted ion chromatograms (XICs) it has been demonstrated that type 2 peptides exhibit a multi peak XIC pattern while linear peptides eluted in a single peak XIC pattern. 2) the assignment of linker fragmentations, which decreases the number of unassigned signals in MS/MS spectra, increase the sequence coverage and the explained ionic current. 3) ¹⁸O labeling, the content of ¹⁸O in type 2 peptides is higher than that of linear peptides. In LC MS/MS analysis of the p 64 K Cys 1 pP 0 p 64 k β Ala 1 pP 0 Bm 86 Cys 1 pP 0 Bm 86 Ac Cys 1 pP 0 and KLH Cys 1 pP 0 conjugates vaccines, more than 85% of the type 2 peptides assigned with the transcyclized and hydrolyzed thiosuccinimide linker eluted with a multi peak XICs pattern. Furthermore, the hydrolyzed thiosuccinimide linker being cleavable in MALDI and ESI MS/MS analyses by metastable gas phase fragmentation generates intense fragment ions P+ 71 and C+ 98 that makes the identification of conjugation sites more reliable. Xion C allows to assign and analyze both these fragment ions and those generated by thioether bond fragmentation (203 P+ 169 C 34) also providing valuable information on the molecular masses of the cross linked peptide pairs. The implementation of these orthogonal methods considerably simplifies the analysis of conjugated vaccines, and speed up the manual validation processes.

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P77

LC-MS/MS characterization of anti-tick conjugate vaccines based on p0 peptide

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Abstract

In our group we have been working in the development of a broad spectrum conjugate vaccine against ticks. These conjugate vaccines were synthesized by using the maleimide-thiol chemistry, where the P0 peptide from *R. sanguineus* was conjugated to three different carrier proteins (Bm86, p64k and KLH) by a thiosuccinimide linker. The conjugation sites are a critical quality attribute of the conjugate vaccines and must be determined. In our strategy, the conjugate vaccine is digested with different proteases, and the complex mixture of proteolytic peptides are analyzed by LC-MS/MS in order to identify the crosslinked peptides (XL-peptides) containing information on the conjugation sites. XL-peptides are composed by two tryptic peptides, one derived from the carrier protein and the other from the antigenic P0 peptide linked by a thiosuccinimide linker and its stabilized forms (transcyclized and hydrolyzed forms). The conjugation sites are identified by using the same software that in cross-linking mass spectrometry experiments assign MS/Ms spectra to XL-peptides. The software output must be validated using the combination of objective and orthogonal criteria that increase confidence in conjugation site assignment. First of all, we used different software that provide redundant and complementary assignment of the conjugation sites. In addition the MS/MS spectra assigned to XL-peptides are validated based on the sequence coverage and the detection of diagnostic ions and linker fragment ions that provide information on the molecular mass of the XL-peptides and the linker form. In addition the linear peptides and XL-peptides showed single- and multi-peak extracted ion chromatograms, respectively. These differences can be used to further differentiate linear and XL-peptides and make the assignment of the conjugation more reliable. The proposed methodology can be used to analyze conjugate vaccines synthesized by either a site-directed and non-site directed conjugation approaches.



CUBA - 2024

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P78

BMPS as a promising cleavable crosslinker for the development and characterization of vaccine conjugates against ticks

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Veterinary vaccines reduce or prevent transmission of zoonotic infections to humans. Developing veterinary vaccine conjugates requires the use of less expensive crosslinkers. Therefore, BMPS (3-Maleimidopropionic acid N-hydroxysuccinimide ester), a heterobifunctional crosslinker, has been used to obtain vaccine conjugates against ticks, showing an efficacy of 54-86% depending on the tick species. These conjugates were obtained by conjugating the Cys1pP0 peptide from the acidic ribosomal P0 protein of *Rhipicephalus* sp to carrier proteins (Bm86, p64k, KLH) using the maleimide-thiol chemistry. Identification of conjugation sites of vaccine conjugates is a requirement requested by the regulatory entities. The aim of this work was to characterize the conjugation sites in Bm86-Cys1pP0, p64k-Cys1pP0 and KLH-Cys1pP0 by LC-MS/MS. The conjugation sites were identified using the stabilized products of the thiosuccinimide linker (transcyclized and hydrolyzed thiosuccinimide linker), as a result of sample processing prior to LC-MS/MS analysis. In Bm86-Cys1pP0, p64k-Cys1pP0 and KLH-Cys1pP0 conjugates, 91, 90 and 74/66 % of Lys residues were partially conjugated to Cys1pP0 peptide. The assignment of crosslinked peptides were validated by the presence of additional diagnostic ions such as P+71 and C+80/C+98, generated by HCD fragmentation of the new pseudopeptide bond in both stabilized crosslinkers. These assignments were also supported by the presence of a two-peak and multi-peak XIC pattern in crosslinked peptides with transcyclized and hydrolyzed thiosuccinimide linkers, respectively. The low cost of BMPS and the presence of validation elements in the assignment of conjugation sites, such as diagnostic ions in MS/MS spectra and multi-peak XIC pattern in crosslinked peptides, make it affordable for the development and characterization of veterinary vaccine conjugates.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P79

Efficacy of the Vaccine Candidate Based on the P0 Peptide against *Dermacentor nitens* and *Ixodes ricinus* Ticks

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Abstract

The control of ticks through vaccination offers a sustainable alternative to the use of chemicals that cause contamination and the selection of resistant tick strains. However, only a limited number of anti-tick vaccines have reached commercial realization. In this sense, an antigen effective against different tick species is a desirable target for developing such vaccines. A peptide derived from the tick P0 protein (pP0) conjugated to a carrier protein has been demonstrated to be effective against the *Rhipicephalus microplus*, *Rhipicephalus sanguineus*, and *Amblyomma mixtum* tick species. The aim of this work was to assess the efficacy of this peptide when conjugated to the Bm86 protein against *Dermacentor nitens* and *Ixodes ricinus* ticks. An RNAi experiment using P0d sRNA from *I. ricinus* showed a dramatic reduction in the feeding of injected female ticks on guinea pigs. In the follow-up vaccination experiments, rabbits were immunized with the pP0-Bm86 conjugate and challenged simultaneously with larvae, nymphs, and the adults of *I. ricinus* ticks. In the same way, horses were immunized with the pP0-Bm86 conjugate and challenged with *D. nitens* larva. The pP0-Bm86 conjugate showed efficacies of 63% and 55% against *I. ricinus* and *D. nitens* ticks, respectively. These results, combined with previous reports of efficacy for this conjugate, show the promising potential for its development as a broad-spectrum anti-tick vaccine.

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P80

Consistency in quality control of the Gavac® immunogen, a successful case.

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ABSTRACT.

Gavac® immunogen, manufactured at the Center for Genetic Engineering and Biotechnology Camagüey, is part of the program integrated control against *Rhipicephalus (Boophilus) microplus*, *R. annulatus* and *R. decoloratus* ticks in cattle (PCIG). In this product, quality control is a fundamental part of the production process, batch release and marketing, both to determine its effectiveness and safety.

The objective of this work is to demonstrate the consistency in the manufacture of the final product evidenced in the quality control of the same. For this, 47 batches manufactured between 2019 and 2023 were analyzed, using the analytical techniques described in the quality specification as a reference.

The physical-chemical and biological characteristics analyzed (thermal and mechanical stability, rheological behavior, droplet size distribution in the emulsion, organoleptic characteristics, sterility and biological activity) demonstrated, through the use of control charts, statistical analysis (variance, coefficient of variation), the consistency of the results obtained in the quality control of the Gavac® immunogen, evidencing a robust production process and compliance with the quality specifications declared in the veterinary health registry in the Republic of Cuba.

Keywords: quality control, Gavac® immunogen, oil emulsions, PCIG.

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P81

Technological update of fermenters and scale increase in the biomass production for the PAI of Gavac®

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The Gavac® immunogen, a veterinary product used to control *R. microplus* infestations, has been produced at the CIGB Camagüey for approximately 20 years. The process runs from the fermentation stage of the yeast expressing the Bm86 protein, to its formulation in oily Montanide. The technological obsolescence of the 50 L and 300 L CHEMAP fermenters, with more than 30 years of operation, and the need to increase the production volumes of the different products manufactured in the CIGB Camagüey multipurpose plant, made their replacement necessary. Therefore, two new fermenters with a capacity of 57 L and 1500 L of total volume were acquired.

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P82

Chemical conjugation scaling up process proposal of the P0 peptide to the Bm86 protein for the production of a broad-spectrum tick vaccine.

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Summary:

The research-scale process to obtain an effective vaccine candidate with broad-spectrum anti-tick action, based on the chemical conjugation between the 20 amino acid peptide of the acidic ribosomal protein P0 of ticks (pP0) obtained by chemical synthesis at the CIGB Havana and the Bm86 protein of *R. microplus* expressed in the yeast *P. pastoris* produced at the CIGB Camagüey as the active pharmaceutical ingredient of the GavacTM vaccine, was transferred to the development stage at the CIGB Camagüey following the protocol approved at the institution. Through this work, we present the scaling up design of the production process of said vaccine candidate, its benefits and its productive capacity.

Keywords: pP0, Bm86, Conjugation, Scaling, Development.

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P83

Obtaining, development and sanitary registration of the veterinary diagnostic product HeberFast Line® GAVAC for use in field conditions.

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The HeberFast Line® Gavac diagnostic system is a tool for the rapid and qualitative evaluation of specific antibodies against the Bm86 antigen in bovine serum. The conditions of the nitrocellulose membrane coating, the conjugation to colloidal gold and the dilution of the samples that allow the use of the diagnostic device were optimized. Likewise, the durability of the strip was determined through a real-time stability study. The consistency of the production process was demonstrated through the production of three batches of strips. The diagnostic sensitivity and diagnostic specificity of this system were above 81% and 91% respectively, and the results were correlated with the ELISA taken as a reference test. All of the above, together with the performance evaluation carried out by a third party, allowed the obtaining of the Sanitary Registration of the diagnostic device granted by the Ministry of Agriculture. For the first time, a veterinary diagnostic device based on lateral flow technology is registered in Cuba.

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P84

Results of vaccination with Gavac within an Integrated Tick Control Program in Cuba

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Abstract

GAVAC® is a subunit vaccine based on the recombinant tick antigen Bm86. It is capable of controlling infestations of *Rhipicephalus microplus*, *Rhipicephalus annulatus*, and *Rhipicephalus decoloratus*. This product is used within an Integrated Tick Control Program (ITCP), wherein the acquired immunity in cattle to Bm86 is combined with a rational use of acaricides and other control tools. The vaccine has been successfully implemented in Cuba to control the level of infestation of the *R. microplus* tick in production farms with dairy or dual-purpose cattle. It has been observed that the vaccine improves animal health and increases livestock production in a more ecological environment. In Cuba, the coordinated efforts of the National Center for Animal Health and the CIGB have facilitated the implementation of an ITCP, resulting in an incremental increase in the number of animals benefiting from the immunogen application each year. The assessment of the principal indicators of the program indicates a reduction in the prevalence of hemoparasitic diseases associated with ticks, the level of infestation by *R. microplus*, and the frequency of tick-killing baths. These findings substantiate the beneficial impact of GAVAC® and the enhancement of cattle quality of life within a healthier environment.

Symposium Livestock anti-tick vaccines and commercialization challenges

Room Guamá, Meliá Internacional Varadero

P85

Enhancing data management efficiency in the integrated vaccination program with GAVAC: A proposal for a multiplatform application.

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Center for Genetic Engineering and Biotechnology

Gavac®, a commercial vaccine against bovine ticks (*Rhipicephalus (Boophilus) microplus*), has been widely adopted in Cuba and exported to several countries in Central and South America. The vaccine's success is evident in Cuba, where over three million cattle have been vaccinated. However, the national vaccination program faces significant challenges in efficiently managing the vast amounts of data generated from production to application. Current data collection and reporting models are inadequate, failing to leverage Information and Communication Technologies (ICTs). This leads to data integrity and availability issues, hindering the generation of reliable reports that inform decision-making. This paper proposes the design of a multiplatform application to integrate, manage, and utilize data from the Gavac® vaccination program more efficiently. The proposed application will offer a range of advantages, including real-time data access, automated reporting, and enhanced data analytics capabilities. By being accessible on multiple devices and platforms, the application will facilitate seamless collaboration and data sharing among stakeholders, improve data accuracy, and enable more informed decision-making. Furthermore, the application's scalability and flexibility will allow it to adapt to the evolving needs of the vaccination program, ensuring a more effective and sustainable response to the challenges posed by bovine ticks.



CUBA - 2024

11TH TTP
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**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

P86

**Description of ticks (Acari: Ixodidae) from Dominican amber below consideration of the recent
genus *Amblyomma***

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Two new fossil ticks from Dominican amber, a larva and an almost fully engorged female are described. Descriptions and assignments to recent genera and species of the fossil ticks, hard and soft ticks have already found in the Dominican amber. Light microscopy images were taken for the morphological description. In addition, high-resolution μ CT scans were carried out with synchrotron radiation at the German Electron Synchrotron (DESY). A precise comparison was carried out using the models subsequently created by editing the 3D digital models. The larva and female have been assigned to genus *Amblyomma*, according to morphological characteristics: anal groove surrounding the anus posteriorly; presence of eyes; hypostome and palps elongate (mainly the article II); spiracular plate comma-shaped. The present study corroborates the previous finding in the literature, that reported for the first time a fossil male tick of the *Amblyomma* genus from American amber.



CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

P87

**Combining morphological and molecular approaches to improve the systematic of the
genus
Hyalomma Koch, 1844**

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Ticks (Acari: Ixodidae) are obligated blood-sucking ectoparasites capable of transmitting a plethora of infectious agents (like viruses, bacteria, and protozoans) to animals, including humans. *Hyalomma* with 27 valid species, is one of the most widely distributed genera, occupying the Afrotropic, Palearctic, and part of the Indomalayan ecoregion. The species of this genus request special attention as they are the main vector of the virus that causes Crimean-Congo hemorrhagic fever in humans, with a 40% fatality rate. However, there are a lot of taxonomic problems in *Hyalomma* regarding the biology and the morphology of the species, as well as sparse sampling, and cryptic speciation that converge in misleading identifications and difficulties in describing the biogeographic patterns of its current diversity. In this study we combine morphology, an exhaustive sampling of the western *Hyalomma* lineages (Southern of Palearctic), and genomic techniques to improve the systematic knowledge of the genus. Our results, combined with available genetic information, show the pattern of distribution of some species, reinforcing the validity of the species with the inclusion of samples from never before collected localities, and improve the evolutionary tree of the genus at a genomic level. Denser taxon sampling across *Hyalomma* distribution, combined with detailed morphological and genomic studies, will improve the systematic knowledge of the genus, which is fundamental for developing measures to control these vectors.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

P88

Molecular characterization of *Ehrlichia canis* TRP36 in thrombocytopenic dogs from Rio de Janeiro, Brazil

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Canine monocytic ehrlichiosis (CME) is a disease caused by the bacterium *Ehrlichia canis*, which is transmitted by the tick *Rhipicephalus sanguineus* sensu lato. Its distribution is more common in regions with a subtropical and tropical climate, being described in all regions of Brazil. The diversity of *E. canis* using the 36kDa tandem repeat protein (trp36) showed four defined genotypes based on the TRP36 gene sequences. In this context, the objective was to determine the genetic diversity of *E. canis* using the TRP36 gene in whole blood samples from naturally infected thrombocytopenic dogs. One hundred and thirty blood samples from thrombocytopenic dogs from the Sul Fluminense mesoregion, in the state of Rio de Janeiro, were used, and were subjected to DNA extraction using the Promega Wizard™ kit. The viability of the extracted DNA was verified by PCR for a fragment of the endogenous GAPDH gene (400bp). Subsequently, molecular analyzes using fragments of the p28 (843bp) and TRP36 (800 to 1000bp) genes were carried out, demonstrating that 15.4% (20/130) of the samples amplified for the p28 gene, specific for *E. canis*. Of these, two samples (10%) amplified for the TRP36 gene and were sequenced for molecular characterization. The two sequences obtained with the TRP36 gene showed a “TEDSVSAPA” tandem repeat pattern belonging to the American genotype with a number of variations of 5 to 7 copies. However, analyzing the amino acids of the complete sequences, it is suggested that they were hybrid strains, that is, formed by the Brazilian genogroup and the American genogroup. It is concluded that the hybrid strain of *E. canis* is circulating in the South Fluminense mesoregion.

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

P89

The European badger (*Meles meles*) as a host for ticks and tick-borne pathogens in peri-urban environments

Short title:

Ticks and tick-borne pathogens from badgers

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Abstract

European badgers, entering peri-urban and urban environments, are opportunistic animals that could serve as important hosts in the life cycle of hard ticks (Acari: Ixodidae). In this study, ticks and spleen samples were collected from badgers (*Meles meles*) which were found as road-kills between 2020 and 2021 in peri-urban habitats in Central Europe, Hungary. Altogether 117 ticks, representing seven species (*Ixodes ricinus*, *Ixodes kaiseri*, *Ixodes canisuga*, *Ixodes hexagonus*, *Haemaphysalis concinna*, *Haemaphysalis inermis* and *Dermacentor reticulatus*) were removed from 49 badgers. Following assessment of suitability for obtaining spleen sample from the carcasses, DNA was extracted and conventional or real-time PCRs were used to detect tick-borne pathogens in tissue samples of 38 badgers. Among protozoan parasites, two *Babesia* species, representing two phylogenetic groups, and a *Hepatozoon* sp. were identified. In addition, *Candidatus* Neoehrlichia lotoris, a novel *Ehrlichia* species (provisionally named as *Candidatus* Ehrlichia transdanubiensis), as well as *Anaplasma phagocytophilum* were detected in these tissue samples. The presence of these tick-borne pathogens in peri-urban mustelids indicate that they may provide a source for the infection of ixodid ticks which can in turn transmit these pathogens to humans or pet dogs in urban habitats. Thus, badgers pose an important epidemiological risk factor at the interface of sylvatic and synanthropic environments.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

P90

Phenotypic and genotypic characterization of ticks and tick-borne pathogens from cattle in the villages

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Abstract

Ticks are blood ectoparasites that feed on domestic, wild animals and humans. They spread a variety of infections such as protozoa, viruses and bacteria. Moreover, cattle reared by smallholder farmers are susceptible to ticks and tick-borne pathogens. Therefore, accurate identification of ticks and detection of tick-borne pathogens is crucial. The aim of this study was to identify and characterise ticks and tick-borne pathogens from selected villages in Greater Letaba municipality, Limpopo province using morphological and molecular techniques. A total of 233 ticks were collected from cattle and identified morphologically using appropriate morphological keys. DNA was extracted from the whole tick for tick identification and from midguts of the ticks for the detection of tick-borne pathogen followed by amplification of 16S rRNA gene before sequencing. The following tick species were morphologically identified from different villages: *Rh. (B) microplus*, *Rh. (B) decoloratus*, *Rh. appendiculatus*, *Rh. evertsi evertsi*, *Rh. sanguineus*, *Am. hebreum*, *Hy. truncatum* and *Hy. rufipes*. *Rhipicephalus spp* was the most common species accounting to 73.8% of the identified ticks. A total of two pathogens were detected from the collected ticks: *Ehrlichia spp* and *Theileria parva* and all the samples tested negative for *Rickettsia* and *Anaplasma*. Congruency between two techniques was tested and the findings show that it was high with a correlation coefficient of 0.857. The findings of this study confirm previous reports indicating that cattle reared by smallholder farmers harbour various ticks and tick-borne pathogens of economic importance. Regular monitoring of tick infestations in the villages around the study areas is recommended to avoid disease outbreaks.



CUBA - 2024

11TH TTP
CONFERENCE

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

P91

**Description of the larva of *Dermacentor latus* Cooley, 1937 (Ixodida: Ixodidae) from two
localities in Costa Rica**

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Dermacentor latus Cooley, 1937 is a neotropical hard tick species, described from Costa Rica and recorded for Panama, with unconfirmed records for Mexico. The adult ticks, both males and females, have been recorded mainly from tapirs, but males have also been found to parasitize dogs. Moreover, human parasitism has been reported for male and female *D. latus*, from only two Panamanian cases. While the male and female have been described, both the nymph and larva of this species remain unknown. Free-living ticks were collected by flagging and direct collection from vegetation in forest trails from November 2023 to March 2024, in two localities in the province of Heredia, Costa Rica, namely San Rafael de Vara Blanca and El Monte de la Cruz, located approximately 1890 and 1910 m.a.s.l., respectively. Two male *D. latus* were identified morphologically, while seven specimens of an unidentified larval morphotype were also collected. A fragment of the cytochrome c oxidase subunit I (*cox-1*) gene was amplified and sequenced for one male *D. latus* and one unidentified larva, resulting in total sequence identity between both specimens. Additionally, a fragment of the 16S *rRNA* gene was also amplified and sequenced for the male. Thus, the unidentified larval morphotype was molecularly identified and hereafter treated as *D. latus*. Four larvae were slide-mounted, morphologically and morphometrically analyzed by light microscopy. Herein we describe the larval morphology of *D. latus* for the first time and provide previously unavailable reference DNA sequence data for the species.

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

P92

A systematic review of ticks and tick-borne pathogens of cattle reared by smallholder farmers in South Africa

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Abstract

Ticks are blood ectoparasites that feed on domestic, wild animals and humans. They spread a variety of infections such as protozoa, viruses and bacteria. Cattle reared by smallholder farmers are susceptible to ticks and tick-borne pathogens due to the type of production system practiced by the farmers. Hence, the focus of this review was mainly on the occurrence of ticks and tick-borne pathogens of cattle reared by smallholder farmers in South Africa. The systematic search produced a total of 13408 articles from four (4) databases, and after screening processes, the review utilized a total of 23 articles that were published between 1983 and 2023. The findings of the review indicated that a total of twenty-six (26) tick species were identified by the reviewed articles, with *Rh. (Bo) decoloratus* and *Rh. evertsi evertsi* being the most prevalent ticks in South Africa. This was followed by *Am. Hebreum*, *Rh. Appendiculatus*, *Hy marginatum rufipes*, *Rh. microplus*, *Rhiphicephalus spp*, *Rh. Follis*, *Rh. gertrudae* and *Hy. truncatum* respectively. In addition, the most reported tick-borne pathogens across the provinces included *B. bigemina*, *B. bovis*, and *A. marginale* respectively; with Eastern Cape province accounting for most of the incidents followed by KwaZulu-Natal and Mpumalanga provinces. The findings of this review confirm that cattle reared by smallholder farmers harbour various ticks and tick-borne pathogens of veterinary, public health and economic importance; and regular monitoring of tick infestations in South Africa is recommended to avoid disease outbreaks.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

P93

Unveiling the genome of *Babesia ovis* Israeli strain: empowering *Babesia* research

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Genome sequencing serves as a powerful tool, offering researchers insight into the genetic intricacies of *Babesia* species. By deciphering their entire genetic blueprint, genes responsible for pathogenicity, drug resistance, and host interaction can be identified. This insight is invaluable for guiding the development of targeted therapies and vaccines to combat *Babesia* infections. Sequencing and annotation of *B. bigemina*, *B. bovis*, *B. caballi*, *B. divergens*, *B. microti*, *B. ovata*, *Babesia* sp. Xinjiang, and recently *B. ovis* genomes have contributed to the development of diagnostic methods and vaccines, as well as screening for drug targets, among others. In the case of *B. ovis* (Selcuk strain), authors have assembled 41 contigs and estimated a genome size of 7.81 Mbp with 3,419 protein-coding genes. This study offers valuable insights into comparative genomics. However, to conduct precise gene editing, it is necessary to consider that genomes from different strains may differ. Therefore, in the present study, the genome of the *B. ovis* Israeli strain was sequenced, assembled, and annotated. For this, an *in vitro* culture of *B. ovis* was established, and genomic DNA was extracted using a commercial Kit. The quality and concentration were assessed on a Qubit 4 Fluorometer. The PacBio Sequel IIe Technology Platform and the HiFi protocol were used to produce highly accurate long reads. With this strategy, 2,920,915 reads were obtained, corresponding to 28.84 Gbp. Preliminary results point to an estimated genome size of 8.58 Mbp. After screening for contamination, the final assembly consisted of nine contigs. The assembly contiguity was confirmed with a contig N50 (Mb) of 2.41 and contig N90 of 1.61. Completeness of the decontaminated assembly was assessed using BUSCO with the Alveolata lineage and revealed 96% complete and single-copy genes. Genome annotation revealed 3,549 genes, with 85.6% having an associated biological function identified based on InterProScan analysis. The obtained genome will be used in future studies aimed at establishing classical and innovative genetic manipulation systems for *B. ovis*.

**Symposium Taxonomy and evolution of ticks and
tick – borne pathogens**

Room Internacional I, Meliá Internacional Varadero

P94

Non-destructive extraction of DNA: a method to keep the shape !

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“My DNA? At the price of my good shape? No way” it is what are thinking all of the ticks when we ask them for more information about their genetic and morphology. Even old specimens from Museum collection (the alcohol-preserved ticks), want to keep their integrity. That is why, to help researchers in their study and to preserve morphology (and mood) of these specimens, non-destructive DNA extraction protocol was applied on *Ixodes ricinus* (larvae, nymphs and adults -female and male) conserved since 2007 in alcohol 70%. The method can extract on average of 69ng of DNA (in a final volume of 30 µl). 16S fragments were successfully amplified and sequenced for all the stages. Photo shoot under stereomicroscope for all of the stages, reassures ticks and researchers that they are keeping their shape but whiten. The contour of the first coxa of larvae, nymphs and adults, a diagnostic character frequently used to discriminate different species was investigated using the outline-based geometric morphometric approach. Our result shows that the coxa 1 is not altered by the DNA extraction. The method is fast, cheap. No body shaming for ticks: the non-destructive DNA extraction preserves the ticks and their morphological characteristics for future reference. It is a valuable aid for integrative taxonomy of ticks.

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

P95

Assessing risk factors and tick-borne pathogens in grazing cattle of northeastern Colombia

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Tick-borne diseases significantly impact cattle herds globally. Ticks in tropical and subtropical regions have been exposed to suitable conditions for continuous multiplication. The cattle ranging in extensive and semi-extensive systems such as in Colombia are continuously infected with tick-borne pathogens, leading to severe health issues and economic losses. In these herds, the transmission and viability of these pathogens could be associated with the risk factors involved in management and environmental variables with scant knowledge of Colombian productions. Because of this, we highlighted in this study the prevalence of tick-borne pathogens and tick infestation incidence impacting the northeastern region of Colombia. We sampled cattle across 21 districts of Cesar including 585 bovines that show a hemoparasites prevalence of 65.1%, revealing 58.0% and 23.5% of prevalence for *Babesia* spp and *Anaplasma* spp, respectively. A high rate (52.6%) of age prevalence was found independently of eatery condition and a small proportion of the cattle (6.6%) showed a high tick infestation incidence. Only 17.6% of the cattle were coinfecting with *Babesia* spp and *Anaplasma* spp. The risk factors for tick-borne pathogens included haematocrit over 45%, reproductive problems on farms, and the movement of animals between farms, and the risk factors for high tick incidences included dairy production, sharing tools and equipment, and the use of personal protection elements by staff. The findings of this study emphasize the need for targeted interventions and management strategies to control hemoparasite infections and tick loads in cattle. By understanding the epidemiological patterns and associated risk factors, stakeholders can implement more effective control measures to mitigate the impact of these diseases on cattle health and productivity. The study underscores the need for targeted interventions to manage and mitigate the impact of tick-borne diseases in cattle. Effective tick control measures, such as regular deworming and tick baths, are essential for managing tick infestations and reducing the prevalence of hemoparasites.

Key Words: Tick-borne diseases, Hemoparasites, *Babesia*, *Anaplasma*, Prevalence, Risk factors.

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CUBA - 2024

11TH TTP
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Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

P96

Coinfection of *Anaplasma bovis*, *Anaplasma* sp. and *Theileria orientalis* complex in European bison (*Bison bonasus*).

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Introduction/objective: The naturalized zoo “Cabarceno Nature Park” (Cantabria, northern Spain) hosts endangered animal species in semi-freedom. Its European bison (*Bison bonasus*) breeding programme, focused on the genetic variability preservation, achieved a herd of 10 animals. Three specimens (two females and one young) dead during 2022 and 2023 without specific symptoms of illness apart from weakness.

Methods: Spleen and blood samples from death bison were analyzed using specific-PCR assays for the tick-borne microorganisms detection: Anaplasmataceae, *Rickettsia*, *Babesia/Theileria/Hepatozoon*, *Trypanosoma*, flaviviruses, orthonairoviruses and phenuiviruses. Subsequently, the detected microorganisms were also searched in blood samples of seven alive bison and 338 questing ticks collected in their facilities.

Results: *Anaplasma bovis*, *Anaplasma* sp. (close to *Anaplasma phagocytophilum*) and *Theileria orientalis* complex (two genotypes) were detected in samples from the three death individuals and in the alive specimens. *Babesia major* was detected in one specimen of the latter. No other microorganisms were identified.

Ticks were grouped in 38 pools: 31 *Haemaphysalis punctata* (n=328), three *Haemaphysalis coccina* (n=5) and four *Ixodes ricinus* (n=5). In *H. punctata* pools, *A. bovis* (n=5), *Anaplasma* sp. (n=1), *T. orientalis* (n=1) and *B. major* (n=4) were detected. Same genotype of *Anaplasma* sp. was also detected in two *H. coccina* pools. *Babesia motasi* and *Theileria capreoli* were amplified from single *H. coccina* pools. Moreover, *A. phagocytophilum* was found in a *I. ricinus* pool.

Conclusions: Coinfections of haemoparasitic tick-borne agents such as *Anaplasma* spp. and *Theileria* spp. should be considered as etiological agents of disease with fatal course in the European bison.

Anaplasma sp. different from *A. phagocytophilum* strains and *Theileria* infection is reported for the first time in European bison.

Haemaphysalis spp. are involved in the epidemiology of the tick-borne pathogens detected in European bison.

Monitoring of ticks and tick-borne pathogens is important for the conservation of endangered species.



CUBA - 2024

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

P97

MOLECULAR DETECTION AND EPIDEMIOLOGICAL ANALYSIS OF *Anaplasma marginale* IN *Rhipicephalus microplus*, *Stomoxys calcitrans* AND *Haematobia irritans*

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Bovine anaplasmosis is a disease caused by the intracellular bacterium *Anaplasma marginale* (Rickettsiales: Anaplasmataceae). It is widespread and can cause significant economic losses. This study investigates the presence of *A. marginale* DNA in the tick *Rhipicephalus* (*Boophilus*) *microplus* and the flies *Stomoxys calcitrans* and *Haematobia irritans* from dairy farms in Seropédica and Paracambi, State of Rio de Janeiro. In the laboratory, the arthropods were dissected and the salivary glands of the ticks and the proboscis of the flies were isolated to extract their DNA using the phenol-chloroform method. They were then analyzed by nested PCR targeting the *msp5* gene. Positive results were obtained for 73% (n=29/40) of *S. calcitrans*, 90% (n=26/29) of *H. irritans* and 45% (n=43/96) of *R. microplus*, which were sequenced by the Sanger method for confirmation, achieving 98.11% to 100% similarity with other *A. marginale* sequences deposited in GenBank. Pairwise comparison showed 97.81% to 100% of similarity between the sequences from this study. A multivariate statistical analysis was then performed to predict the factors associated with *A. marginale* infection in *R. microplus* using multiple logistic regression analysis. The presence of flies over the animals was considered as a risk factor, with infested animals being 3 times more likely to have their ticks infected with *A. marginale* (p-value= 3.26; OR:3.26; IC:1.17-9.08). This study is preliminary and highlights the possibility of these hematophagous dipterans being the main vectors of *A. marginale* in the epidemiological chain of the disease. It also highlights the importance of experimental research and the implementation of new strategies to prevent all possible vectors of this pathogen that affects so many cattle around the world.



CUBA - 2024

11TH TTP
CONFERENCE

Symposium Taxonomy and evolution of ticks and tick – borne pathogens

Room Internacional I, Meliá Internacional Varadero

P98

Insights and methodological approaches for exploring hemoglobin metabolism in Babesia parasites

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Babesia is a genus of parasitic protozoa that infects red blood cells in various animals, including humans, and is transmitted through *Ixodes ricinus* ticks. Its prevalence in Europe has considerable effects on the livestock sector, posing substantial challenges to the health and productivity of cattle.

Extensive research efforts have been dedicated to unraveling the intricacies of hemoglobin metabolism in *Babesia* parasites. The research encompasses diverse analytical techniques, ranging from HPLC-MS for haem degradation products to cryo-immuno electron microscopy for elucidating hemoglobin's role in the parasite's lifecycle. It involves visualizing intracellular trafficking of host hemoglobin in *Babesia*, identifying proteins involved in hemoglobin processing, and screening inhibitors impairing iron and haem metabolism.

To validate hemoglobin as the primary nutrient source for intraerythrocytic *Babesia* parasites and to determine connected haem utilization and degradation pathways, several steps were taken. Parasites were cultured using gold-labeled hemoglobin, and electron scanning microscopy confirmed hemoglobin internalization. *Babesia* parasites were then incubated with the fluorescent haem analogue to assess uptake and intracellular trafficking.

Whole-cell proteomic analysis compared parasites grown under control conditions with those exposed to the iron chelator DFO. Proteins implicated in iron homeostasis were identified and chosen for characterization and functional studies.

LC-MS/MS quantified biliverdin levels within cellular extracts, revealing significant differences between parasitized and uninfected RBC cultures. The results indicate that *B. divergens* infection leads to notable changes in biliverdin levels within RBCs due to enzymatic haem degradation within parasites.

Overall, these methodologies shed light on hemoglobin's crucial role as a nutrient source for *Babesia* parasites and elucidate associated haem utilization and degradation pathways.

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Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

P99

Investigating the role of miRNAs in the salivary glands of the soft ticks *Ornithodoros moubata* and *Ornithodoros erraticus*

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MicroRNAs (miRNA) are small non-coding RNAs known to modulate gene expression by inhibiting or degrading messenger RNA (mRNA). In the context of tick saliva, miRNAs are emerging as key players in interactions between ticks and their hosts. We previously demonstrated the presence of miRNAs in the saliva of *Ornithodoros erraticus* (Oe) and *Ornithodoros moubata* (Om), primary vectors of African swine fever (ASF) and human relapsing fever (TBRF) in the Mediterranean Basin and Southeastern Africa, respectively. *In silico* studies confirmed that saliva miRNAs could be involved in various host processes, such as immune response, gene expression and vascular development, while their role in *Ornithodoros* tick physiology remains unclear. This study aims to explore the role of salivary miRNAs in regulating physiological processes in the salivary glands of Oe and Om ticks. Using the miRbase database and our previous dataset on saliva miRNAs, we identified conserved isomiR sequences in both species, which were subsequently grouped into miRNA families. Further analysis focused on mature sequences present in all replicates, resulting in 141 miRNA sequences in Oe and 86 in Om. These miRNA sequences were employed for *in silico* analysis of their role in salivary gland physiology, specifically targeting the predicted 3'UTR regions from the transcriptomes of both species. In Oe, *in silico* predictions showed 3,378 unique targets primarily associated with positive regulation of exocytosis, intracellular mRNA localization and signal transduction, while in Om, 4,223 targets were mostly related to carbohydrates metabolism and regulation of multicellular organism development. These findings underscore the significant role of miRNAs in regulating physiological processes in tick salivary glands, providing insights into tick-host interactions and potential targets for future intervention strategies.

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Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

P100

Detection of multiple novel viruses in hard and soft ticks in Mexico

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Abstract

We examined ticks from Mexico using viral metagenomics to increase our understanding of the composition and diversity of the tick virome. The analysis was performed using 3131 ticks of different species of Ixodidae and Argasidae collected in 2019-21 from domestic animals in four states of Mexico (Chiapas, Chihuahua, Guerrero, and Michoacán). All ticks were homogenized and tested for viruses using two approaches. In the first, an aliquot of each homogenate underwent two blind passages in *Ixodes scapularis* cells. Supernatants from all second passage cultures were subjected to polyethylene glycol precipitation to enrich for virions then RNAs were extracted from the precipitates and analyzed by unbiased high-throughput sequencing. In the second, an aliquot of every homogenate was subjected to PEG precipitation then RNAs were extracted and analyzed by UHTS, allowing for the detection of viruses unable to replicate in ISE6 cells. We identified seven novel viruses from multiple taxonomic groups (Bunyavirales, Flaviviridae, Nodaviridae, Nyamiviridae, Rhabdoviridae, Solemoviridae, and Totiviridae), some of which are highly divergent from all classified viruses and cannot be assigned to any established genus. Twelve recognized species of viruses were also identified.

Keywords: ticks, virus discovery, metagenomics, RNA-seq, high-throughput sequencing, Mexico.

Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

P101

Tick Genomics – Progress Towards Tick Genome Assembly and Annotation Best Practices

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Ticks are important disease vectors for humans and animals, resulting in significant monetary losses annually in healthcare and veterinary costs. Approximately 900 tick species have been described, however, only 14 reference genomes are available on NCBI database. Tick reference genomes have been used to understand tick-host-pathogen interactions, pesticide resistance, and potential vaccine targets. Tick genomes have been challenging to generate as they are similar in size to vertebrate, mammalian genomes, have high amounts of biological contamination, and contain a large proportion and diversity of repetitive elements. While the recent development of long-read sequencing has enabled the generation of high-quality tick genome assemblies, best practices remain unidentified. Here, we describe whole genome assembly for two European tick species: *Dermacentor reticulatus* and *Ixodes ricinus*.

Ticks were collected in the Netherlands and DNA extracted from three individual adult females per species. Each DNA sample was individually prepared and sequenced in parallel on an Oxford Nanopore PromethION and Illumina NovaSeq 6000. Long-reads from all runs for each individual tick were characterized via Nanoplot, assembled via Flye, and heterozygous diploid genome contigs purged via purge_haplotigs. The *I. ricinus* basecall errors were polished using short-reads via NextPolish, and contamination removed using Foreign Contamination Screen and bedtools. All assemblies were characterized with QUAST, BUSCO, and/or compleasm. Annotation was performed on *I. ricinus* using a homology-based approach via Liftoff using the annotation of *I. scapularis* as a template, and repetitive elements were characterized via a custom pipeline.

We generated high-quality assemblies for both tick species that were comparable to other tick genome assemblies without amplification. When the ONT data from the *I. ricinus* individual with the most data were filtered most stringently, the resulting assembly quality improved, but contained extraneous satellite sequences. ONT provides an efficient route to full-genome assemblies of individual ticks, with carefully characterized repetitive elements.

Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

P102

Karoo Paralysis tick (*Ixodes rubicundus*) salivary gland transcriptomes – in search of a toxin

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Certain ticks induce ascending paralysis during feeding. With no effective treatment, understanding the toxins responsible for tick paralysis is crucial for developing control measures. In South Africa, *Ixodes rubicundus* poses a significant threat to livestock and wildlife. The current study analysed the nymphal salivary gland transcriptome of unfed, early, and late paralysis phases, identifying over 35,000 contigs. Many contigs lacked functional annotation, emphasizing the need for further research. Secretory protein expression, especially lipocalins, Kunitz-domain proteins, TIL-domain proteins, and basic tail secretory proteins, increased during feeding, mirroring a paralysis strain of *Rhipicephalus evertsi evertsi*. Extracellular matrix-like proteins were highly expressed, suggesting exoskeleton remodelling and adhesion during feeding. Similarities were noted between the current transcriptome and a toxic fraction of metastriate paralysis transcriptome, particularly the 5.3 kDa protein family. However, higher expression of ETX_MTX2 and metalloproteases reprotysin and gluzincin were observed. Interestingly, these components resemble toxin constituents found in wasps, centipedes, spiders, and snakes, impacting the extracellular matrix or feeding site. These components are also present in non-paralysis tick transcriptomes. Tick transcriptomes will assist research into tick toxins and their functional significance to mitigate the impact of tick-borne diseases on both human and animal populations.



CUBA - 2024

Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

P103

Bacteriome Diversity in *Babesia ovis*-Infected *Rhipicephalus bursa* salivary glands and midguts

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Ticks, like all living organisms, host a diverse microbial community that can impact numerous facets of tick biology. One of the most studied aspects of microbiomes is their potential impact on the acquisition, establishment, and transmission of pathogens. There are several modes by which the bacteriome can influence pathogen transmission. For instance, some bacteria in the tick's microbiome can outcompete tick-borne pathogens for resources or produce substances that hinder or promote the establishment of pathogens. Additionally, they may interact with the tick's immune system, either enhancing or suppressing immune responses. Therefore, the present study aims to characterize the microbiota of both uninfected and *Babesia ovis*-infected *Rhipicephalus bursa* ticks, a ubiquitous vector of ovine babesiosis. For this study, adult female ticks from both uninfected and *B. ovis*-infected *R. bursa* colonies, previously established at CEVDI/INSA, were used. Salivary glands and midguts from individual ticks were isolated, and DNA was extracted using a commercial kit. Infection was confirmed by PCR, and twelve samples comprising three replicates per tissue and condition were prepared. The conserved bacterial 16S hypervariable regions (V3-V4) were sequenced using MiSeq Illumina, employing a pairwise alignment sequence dissimilarity approach. The Minimum Entropy Decomposition (MED) algorithm was used for operational taxonomic unit (OTU) identification, and further assignments were performed using the QIIME software package. A total of 1,525 OTUs were identified among the 12 samples analyzed. The bacterial composition was quite variable in each experimental group. At the species level, the analysis of taxa revealed that *Sphingomonas* constitutes the largest proportion of the microbiome in midguts and salivary glands in uninfected specimens. Following *B. ovis* infection, a lower amount of *Sphingomonas* taxa was observed in infected tissues. Ultimately, this data will aid in the understanding of the complex interactions between ticks, pathogens, and their resident bacteria and can be exploited to develop new effective strategies for tick and tick-borne disease control.



CUBA - 2024

11TH TTP
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Symposium Tick microbiome and genomics

Friday September 6th, Room Internacional II, Meliá Internacional Varadero

P104

Title: Understanding the vertical and horizontal transmission potential of *Spiroplasma* in ticks

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Many arthropods harbour bacterial symbionts, which are maintained by vertical and/or horizontal transmission. *Spiroplasma* is one of the most well-known symbionts of arthropods including ticks. Based on the phylogenetic analyses, *Spiroplasma* may infect ticks via both vertical and horizontal transmission, although there is no direct observation in the laboratory settings. The aim of this study is to clarify the potential routes of transmission of *Spiroplasma* in its own tick hosts.

First, PCR screening was conducted to detect *Spiroplasma* spp. in questing ticks (n = 712) collected from diverse geographic regions in Japan. All PCR amplicons were sequenced to analyse the phylogenetic relatedness with host tick species. Second, the vertical transmission of *Spiroplasma* detected in *Ixodes ovatus* adults was investigated by detecting *Spiroplasma* in the eggs and larvae by experimentally infesting on a rabbit. Third, isolates of tick-derived *Spiroplasma* were experimentally inoculated into *Spiroplasma*-free *Haemaphysalis longicornis* adults, and the



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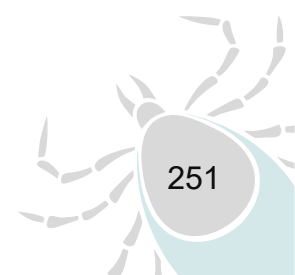
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CUBA - 2024

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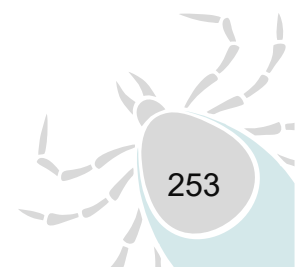
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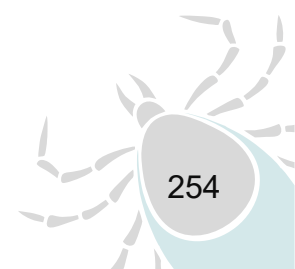
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