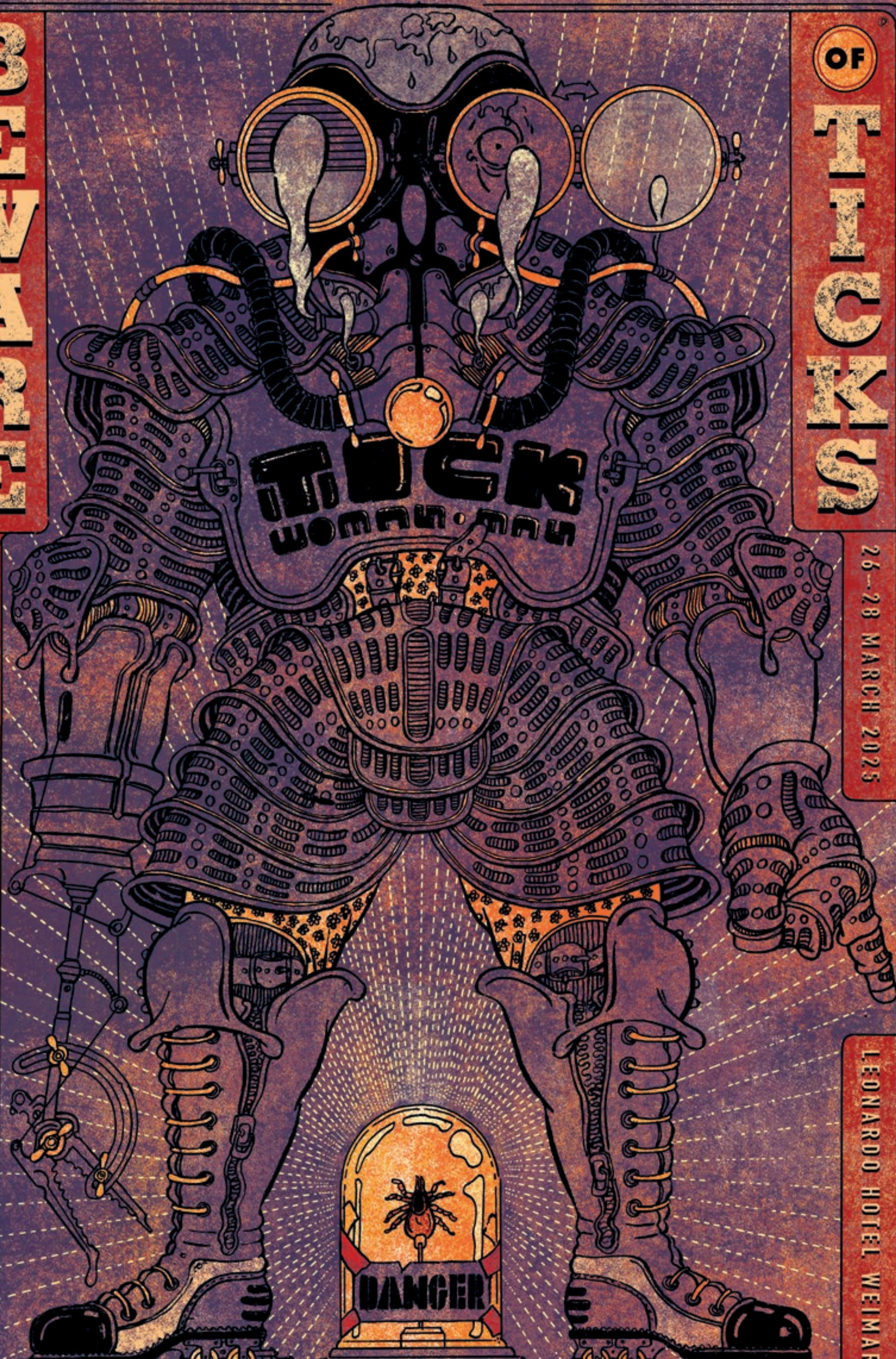
BEWARE OF TICKS

XVIth INTERNATIONAL SYMPOSIUM ON TICKS AND TICK-BORNE DISEASES

26–28 MARCH 2025

LEONARDO HOTEL WEIMAR



© btiger | AdobeStock

TABLE OF CONTENTS ISTTBD-XVI

Organisation and imprint 4

Welcome note 5

Programme overview 6

Scientific programme

Wednesday, 26 March 7
Thursday, 27 March 11
Friday, 28 March 18

Poster presentations

Thursday, 27 March 22
Friday, 28 March 28

Sponsors and exhibitors 35

Floor Plan 36

Social and cultural programme 37

General information 38

General information for online participation 41

Index of authors and chairs 42

Abstracts 44

DESIGN/LAYOUT ISTTBD-XVI

Layout	Conventus Congressmanagement und Marketing GmbH
Print	printworld.com GmbH I Messering 5 I 01067 Dresden
Editorial Deadline	10 March 2025

© Layout Cover Robert Voss

ISTTBD-XVI ORGANIZATION AND IMPRINT

Date
26–28 March 2025

Conference website
www.ittbd-symposium.com

Hybrid symposia
www.ittbd-symposium-digital.com

Conference chairs
Prof. Dr. Ard Nijhof
Freie Universität Berlin
Institute for Parasitology and Tropical Veterinary Medicine
Co-Editor-in-Chief of Ticks and Tick-borne Diseases (Berlin/DE)

Dr. Olaf Kahl
tick-radar GmbH
Managing Editor of Ticks and Tick-borne Diseases (Berlin/DE)

Prof. (a.D.) Dr. Jochen Süss
BREHMS WELT – Tiere und Menschen
Former-Editor-in-Chief of Ticks and Tick-borne Diseases (Renthendorf/DE)

Scientific committee
Gerhard Dobler (Bundeswehr Institute of Microbiology)
Ute Mackenstedt (Universität Hohenheim)
Cornelia Silaghi (Friedrich-Loeffler-Institut)

Professional congress organiser
Conventus Congressmanagement & Marketing GmbH
Claus Winkler
ticks-symposium@conventus.de
www.conventus.de

Dear Colleagues,

It is our great pleasure to welcome you to the 16th International Symposium on Ticks and Tick-borne Diseases (ISTTBD-XVI)!

Building on the success of our previous hybrid symposium, we decided to once again offer both in-person and virtual participation. This format enables valuable face-to-face interactions while allowing colleagues from around the world to join us remotely. We encourage you to make the most of this opportunity, whether by engaging in discussions on-site, participating and networking online, or providing feedback on how we can continue to improve this experience.

We are again thrilled by the large number of abstract submissions this year. They form the heart of our symposium, and while time constraints limited the number of oral presentations that could be accommodated, we deeply appreciate the breadth of research being shared. We look forward to lively discussions and knowledge exchange across all sessions.

At the same time, we acknowledge that science and scientific collaboration are increasingly facing challenges. Political and economic pressures, restrictions on academic freedom, and barriers to international cooperation are affecting many of our colleagues. It remains important to foster open dialogue, uphold the principles of scientific integrity, and strengthen our global community. By coming together at events like ISTTBD-XVI, we reaffirm our commitment to advancing knowledge, supporting one another, and ensuring that scientific progress continues despite these obstacles.

This year’s program is set to be both stimulating and inspiring, featuring five keynote lectures and 60 oral presentations, including a special session where six Early Career Researchers will compete for the prestigious Sinnecker-Kunz award. Additionally, the program includes 26 flash talks, two extended poster sessions, and a panel discussion on scientific publishing. We are also pleased to once again showcase the artistic work of Robert Voss, who has designed this year’s symposium poster. We extend our sincere gratitude to our main sponsors, Pfizer, Bavarian Nordic, and Elanco, whose generous support has made this event possible.

We wish all participants a rewarding and engaging symposium filled with meaningful discussions, new collaborations, and shared enthusiasm for research on ticks and tick-borne disease research. Let’s make ISTTBD-XVI a memorable and impactful experience!

Yours,

Jochen Süss, Olaf Kahl, Ard Nijhof
Conference chairs, ISTTBD-XVI

ISTTBD-XVI SCIENTIFIC PROGRAMME

26.03.2025

Goethesaal

10:30–10:45
Opening

10:45–11:15
Keynote 1

11:20–12:15
Session 1
Borrelia

12:20–13:00
Session 2
Field ecology I

14:00–14:50
Session 3
Field ecology II

14:55–16:20
Award session
Sinnecker-Kunz Award

16:50–17:20
Keynote 2

17:25–18:15
Session 4
The microbiome of Idoxes ticks

18:15–18:50
Session 5
Ticks – genomic insights and antimicrobiota vaccines

19:30–21:30
Welcome Reception

27.03.2025

Goethesaal

08:50–09:20
Keynote 3

09:20–09:45
Panel Discussion

09:50–11:00
Session 6
Tick-borne Encephalitis

11:30–12:10
Poster flash talks I

14:10–14:40
Keynote 4

14:40–15:20
Session 7
Lyme Borreliosis

15:20–16:05
Session 8
Evolution and systematics

16:35–17:15
Session 9
Tick saliva and salivary grands

17:15–17:55
Session 11
Tick breeding and climate impact on pathogen dynamics

17:55–18:35
Session 13
Pathogen genomics and tick control

Forum

16:35–17:15
Session 10
The brown dog tick(s)

17:15–17:55
Session 12
Ticks and tick-borne diseases of cattle

17:55–18:35
Session 14
Tick-borne protozoa

Belvedere

12:10–13:10
Poster session 1

28.03.2025

Goethesaal

09:00–10:20
Poster flash talks II

11:50–12:20
Keynote 5

12:20–13:10
Session 15
Tick-host interactions & pathogen transmission dynamics

13:10–14:30
Session 16
Insights into tick physiology, immunomodulation and microbe interactions

14:30–15:00
Awards and Closing

Belvedere

10:20–11:50
Poster session 2

Poster session
Social programme
Keynote session
Short lectures
Registration/opening/closing

10:30–10:45	Opening address
Room	Goethesaal
10:45–11:15	Keynote I
Room	Goethesaal
Chairs	P. Kraiczy (Frankfurt/DE), K. Strle (Boston/US)
10:45	OP 001 Immune response in the pathogenesis of Lyme disease K. Strle (Boston/US)
11:10	Discussion
11:20–12:15	Session I | *Borrelia*
Room	Goethesaal
11:20	OP 002 The completed genome of *Borrelia tillae* – a relapsing fever *Borrelia* species from South Africa G. Margos (Oberschleissheim/DE)
11:30	OP 003 *Borrelia tillae*, a relapsing fever species from South Africa with human pathogenic potential P. Kraiczy (Frankfurt/DE)
11:45	Discussion
11:50	OP 004 A core genome MLST scheme for *Borrelia burgdorferi* sensu lato improves insights into the evolutionary history of the species complex S. Hepner (Oberschleissheim/DE)
12:00	OP 005 Characterisation of *Borrelia burgdorferi* sequence diversity using modified multilocus sequence typing O. N. Choi (Orono/US)
12:10	Discussion

12:20–13:00	Session II | Field ecology I
Room	Goethesaal
Chairs	N. Boulanger (Strasbourg/FR), V. Hönig (České Budějovice/CZ)
12:20	OP 006 Tickborne pathogen prevalence of small mammals in Pennsylvania S. Schwartz (East Stroudsburg, PA/US)
12:30	OP 007 Analysis of ticks (Order: Ixodida) and associated microorganisms collected on the North Sea Island of Heligoland R. E. Rollins (Wilhelmshaven/DE)
12:40	OP 008 Impact of extreme weather events on tick-host dynamics and disease risk in mountainous regions – the study case of Vaia windstorm in the north eastern Italian Alps V. Tagliapietra (San Michele all'Adige/IT)
12:50	Discussion
13:00–14:00	Lunch snack | Belvedere
14:00–14:50	Session III | Field ecology II
Room	Goethesaal
14:00	OP 009 Ticks and tick-borne pathogens in urban areas in the Czech Republic V. Hönig (České Budějovice/CZ)
14:10	OP 010 New patterns in seasonal activity of two epidemiologically important ticks in Slovakia, *Ixodes ricinus* and *Dermacentor reticulatus*, and their infection with tick-borne agents M. Kazimírová (Bratislava/SK)
14:20	OP 011 Unravelling urban tick-borne pathogen infection risk – are rodents key reservoirs in urban settings? J. Rapp (Hannover/DE)
14:30	OP 012 Acarological risk in urban and peri-urban ecosystems in a French region highly endemic for ticks and tick-borne diseases N. Boulanger (Strasbourg/FR)
14:40	Discussion

14:55–16:20	Sinnecker-Kunz Award
Room	Goethesaal
Chairs	C. Strube (Hannover/DE), M. Pfeffer (Leipzig/DE)
14:55	Introduction
15:00	OP 013 Kinin neuropeptide play a broad diversity of biological functions in *Ixodes ricinus* physiology L. Abiun-Denis (Maisons-Alfort/FR)
15:10	OP 014 African swine fever virus and vector competence in soft ticks – What do we need to do to unravel the unknown? T. Lao-Gonzalez (Havana/CU)
15:20	OP 015 Tick-borne viral pathogens in ticks and human patients from the Czech Republic J. Kamiš (České Budějovice/CZ)
15:30	OP 016 Female mice infected with *Borrelia burgdorferi* transmit maternal antibodies to their offspring that provide strain-specific protection against tick bite A. Foley-Eby (Saskatoon/CA)
15:40	OP 017 Artificial feeding of ticks – a systematic review B. E. Mustafa (Werribee/AU)
15:50	OP 018 Establishing of an artificial tick feeding system as contribution to the 3Rs approach – promising results obtained with feeding medically relevant tick species K. Elati (Berlin/DE)
16:00	Discussion
16:20–16:50	Coffee break | Belvedere

16:50–17:20	Keynote II
Room	Goethesaal
Chairs	A. Cabezas Cruz (Paris/FR), J. J. Khoo (Liverpool/UK)
16:50	OP 019 Tick-borne relapsing fever spirochetes in South America: past, present and future directions S. A. Muñoz Leal (Concepción/CL)
17:15	Discussion
17:25–18:15	Session IV | The microbiome of *Ixodes* ticks
Room	Goethesaal
17:25	OP 020 *Midichloria mitochondrii*, a symbiont of *Ixodes ricinus* ticks, contributes to Lyme disease risk A. I. Krawczyk (Esch-sur-Alzette/LU)
17:40	OP 021 Exploring *Ixodes ricinus* endo-microbiome transmission modes P. Lesiczka (Wageningen/NL)
17:55	OP 022 Complex interactions between two lineages and two symbionts in *Ixodes frontalis* S. Melis (Pavia/IT)
18:05	Discussion
18:15–18:50	Session V | Ticks – genomic insights and anti-microbiota vaccines
Room	Goethesaal
18:15	OP 023 Innovative approaches to vector vaccination – targeting the microbiota to prevent vector-borne diseases A. Cabezas Cruz (Paris/FR)
18:30	OP 024 Near-complete bacterial genome insertion in a tick nuclear genome evidence from tick cell lines and ticks J. J. Khoo (Liverpool/UK)
18:45	Discussion
19:30–21:30	Welcome Reception | Belvedere

08:45–08:55	Announcements & Introduction
Room	Goethesaal
08:55–09:20	Keynote III
Room	Goethesaal
Chairs	J. de la Fuente (Ciudad Real/ES), O. Kahl (Berlin/DE)
08:55	OP 025
	Academic publishing: doom – Or dawn of a new era?
	U. Dirnagl (Berlin/DE)
09:20–09:45	Panel Discussion | Scientific Publishing
Room	Goethesaal
09:50–11:00	Session VI | Tick-borne Encephalitis
Room	Goethesaal
Chairs	A. Rizzoli (San Michele all'Adige/IT), M. Palus (České Budějovice/CZ)
09:50	OP 026
	Pericytes and microglia in tick-borne encephalitis pathogenesis and blood brain barrier dynamics
	M. Palus (České Budějovice/CZ)
10:05	OP 027
	Chemokine-binding proteins in *Ixodes ricinus* saliva
	J. Kotál (České Budějovice/CZ)
10:15	Discussion
10:20	OP 028
	Changes in the genetic structure of population of the three tick-borne encephalitis virus strains representing the main virus subtypes during persistence in different tick species
	A. Polienko (Moscow/RU)
10:30	OP 029
	A spatio-temporal modeling framework to assess the probability of human tick-borne encephalitis (TBE) infections across Europe
	F. Dagostin (San Michele all'Adige/IT)
10:40	OP 030
	Thermosensitivity of TBE virus strains – experimental studies
	A. Lindau (Stuttgart/DE)
10:50	Discussion

11:00–11:30	Coffee break | Belvedere
11:30–12:10	Flash talks I
Room	Goethesaal
Chairs	A. Zintl (Dublin/IE), V. Fingerle (Oberschleissheim/DE)
11:30	PP 022 Results from eight years of surveillance of ticks and tick-borne encephalitis virus in southern Norway A. P. Cotes Perdomo (Oslo/NO)
11:35	PP 002 Evolution of the *vls* antigenic variation system in Lyme *Borrelia* genomes A. Collado (New York, NY/US)
11:40	PP 023 Establishing multicellular blood-brain barrier infection models for antiviral drug discovery M. Zuniga (Oberschleissheim/DE)
11:45	PP 025 Studies on the co-localisation of TBE-virus and *Borrelia* species at selected TBEV-foci in Baden-Wuerttemberg R. Vogt (Stuttgart/DE)
11:50	PP 026 Tick-borne encephalitis virus NS1 disrupts the integrity of endothelial monolayer M. Čížková (České Budějovice/CZ)
11:55	PP 029 Quasispecies in tick-borne encephalitis virus – utilising long-read next generation sequencing to determine population diversity and genomic plasticity in tick-borne flaviviruses L. Tardy (České Budějovice/CZ)
12:00	PP 015 Update on tick-borne pathogens and new insights of the microbiota of hard ticks in Portugal L. Moerbeck (Lisbon/PT)
12:05	PP 033 Identifying primary and alternative tick vectors for Crimean-Congo hemorrhagic fever virus: the importance of defining vectorial capacity of ticks for human risk D. Bente (Galveston/US)

12:10–13:10	Poster session I and lunch snack | Belvedere
14:10–14:40	Keynote IV
Room	Goethesaal
Chairs	G. Margos (Oberschleissheim/DE), A. Moniuszko (Bialystok/PL)
14:10	OP 031 Tick-borne pathogens in Poland from a clinical perspective A. Moniuszko (Bialystok/PL)
14:35	Discussion
14:40–15:20	Session VII | Lyme Borreliosis
Room	Goethesaal
14:40	OP 032 Impact of acute Lyme disease in children L. Nigrovic (Boston, MA/US)
14:50	OP 033 KillaRay – a novel approach preventing *Borrelia afzelii* amplification in human skin model D. Wężyk (Warsaw/PL)
15:00	OP 034 Duplex PCR assay to determine sex and mating status of *Ixodes scapularis*, vector of the Lyme disease pathogen I. Ronai (Cambridge, MA/US)
15:10	Discussion

15:20–16:05 Room Chairs	Session VIII | Evolution and systematics Goethesaal L. Chitimia-Dobler (Munich/DE), B. J. Mans (Onderstepoort/ZA)
15:20	OP 035 From phylogenomics to functional evolution – insights into the adaptation of ticks to a blood-feeding environment using comparative sialomics B. J. Mans (Onderstepoort/ZA)
15:35	OP 036 Redescription of *Alloceraea cretacea* with an additional nymphal fossil added to this species L. Chitimia-Dobler (Munich/DE)
15:45	OP 037 Similarities between *Ixodes ricinus* and *Ixodes inopinatus* genomes and horizontal gene transfer from their endosymbionts O. Jlassi (Utrecht/NL)
15:55	Discussion
16:05–16:35	Coffee break | Belvedere
16:35–17:15 Room Chairs	Session IX | Tick saliva and salivary glands Goethesaal H. Dautel (Berlin/DE), J. Perner (České Budějovice/CZ)
16:35	OP 038 Octopamine and adrenergic-like octopamine receptors in *Ixodes ricinus* salivary glands S. Rakotobe (Maisons Alfort/FR)
16:45	OP 039 A sTicky Situation – on the phase separation and ageing of disordered proteins from tick cement cone S. Deshpande (Wageningen/NL)
16:55	PP 017 Identification of ion transport peptide-like (ITPL) signalling in the tick *Ixodes ricinus* V. Klöcklerová (Bratislava/SK)
17:00	PP 018 Enzymes beyond tick midgut hemolysis T. Kozelková (České Budějovice/CZ)
17:05	Discussion

16:35–17:15	Session X | The brown dog tick(s)
Room	Forum
Chairs	G. Klafke (Eldorado do Sul/BR), S. Kelava (Sapporo/JP)
16:35	OP 042 Are there 16 species of brown dog ticks? Phylogenies from 59 entire mitochondrial genomes and 157 *cox1* sequences reveal 16 species-level clades in the *Rhipicephalus (Rhipicephalus) sanguineus* group S. Kelava (Sapporo/JP)
16:45	OP 043 Laboratory crosses of *Rhipicephalus sanguineus* s.s. and *Rhipicephalus hibericus* demonstrate lack of fertile progeny and support species separation A. Estrada-Peña (Zaragoza/ES)
16:55	OP 044 *Rhipicephalus sanguineus* sensu stricto (Latreille, 1806) and *Rhipicephalus linnaei* (Andouin, 1826) – Are they also different in susceptibility to acaricides? G. Klafke (Eldorado do Sul/BR)
17:05	Discussion
17:15–17:55	Session XI | Tick breeding and climate impact on pathogen dynamics
Room	Goethesaal
Chairs	H. Dautel (Berlin/DE), J. Perner (České Budějovice/CZ)
17:15	OP 045 Laboratory breeding of *Ixodes hexagonus* – a tick breeder`s perspective H. Dautel (Berlin/DE)
17:25	OP 046 The niche of *Borrelia burgdorferi* s.l. in Europe is predictable and mappable A. Estrada-Peña (Zaragoza/ES)
17:35	OP 047 The spatial range of *Borrelia burgdorferi* s.l. in Europe under a future scenario of +2°C. P. Kelly (New York, NY/US)
17:45	Discussion

17:15–17:55	Session XII | Ticks and tick-borne diseases of cattle
Room	Forum
Chairs	G. Klafke (Eldorado do Sul/BR), S. Kelava (Sapporo/JP)
17:15	OP 048 Insights into outbreaks of *Rhipicephalus microplus* ticks in the cattle tick-free zone in Southern Brazil J. Reck (Eldorado do Sul, RS/BR)
17:25	OP 049 Structural analysis of Bm86-homolog anti-tick vaccine antigens E. Jacobs (Queens, NY/US)
17:35	OP 050 Molecular characterisation of *Theileria* species in bovine theileriosis in Zimbabwe V. Pinarello Kleiber (Harare/ZW)
17:45	Discussion
17:55–18:35	Session XIII | Pathogen genomics and tick control
Room	Goethesaal
Chairs	H. Dautel (Berlin/DE), J. Perner (České Budějovice/CZ)
17:55	OP 051 Genomic characterisation of UK *Anaplasma phagocytophilum* strains and optimisation of metagenomic enrichment protocols for whole genome sequencing of *A. phagocytophilum* directly from infected tissues R. Birtles (Salford/UK)
18:05	OP 052 RoaM affects the transcription of several putative effectors in *Rickettsia rickettsii* governing the transition to a cellular program of active spreading A. Nock (Bryan/US)
18:15	OP 053 Using machine learning to discover novel tick repellents and acaricides M. Vlot (Nijmegen/NL)
18:25	Discussion

17:55–18:35	Session XIV | Tick-borne protozoa
Room	Forum
Chairs	G. Klafke (Eldorado do Sul/BR), S. Kelava (Sapporo/JP)
17:55	OP 054 Characterization of secreted *Theileria annulata* effector protein Ta9 S. Tajeri (Berlin/DE)
18:05	OP 055 Moving beyond the 18s rRNA gene as a diagnostic target for *Babesia microti* T. F. Rounsville Jr. (Orono/US)
18:15	OP 056 Babesiosis as an emerging concern in the Mid-Atlantic United States: Autochthonous human babesiosis cases and *Babesia microti* (Piroplasmida: Babesiidae) in *Ixodes scapularis* (Acari: Ixodidae) and *Ixodes keiransi* (Acari: Ixodidae) ticks from Delaware, Maryland, Virginia and West Virginia, 2009–2024 R. Nadolny (Edgewood, MD/US)
18:25	Discussion
19:30	Social Evening | Villa Haar

09:00–10:15	Flash talks II
Room	Goethesaal
Chairs	A. Estrada-Peña (Zaragoza/ES), S. Cutler (London/UK)
09:00	PP 055 Innovative machine learning algorithms to understand the driving forces for tick abundance across Europe S. Lansdell (London/UK)
09:05	PP 077 *Borrelia burgdorferi* s.l. in ticks from Lyme neuroborreliosis-risk areas in Denmark N. Król (Leipzig/DE)
09:10	PP 059 The survivability of *Rhipicephalus sanguineus* s.s. and *Rh. linnaei* in Germany: a controlled outdoor study K. Fachet-Lehmann (Stuttgart/DE)
09:15	PP 050 Improvement of the serodiagnosis of tick- and louse-borne relapsing fever F. Reyer (Frankfurt/DE)
09:20	PP 063 Utilising retrotransposon blood meal analysis (Rt-BMA) to identify *Ixodes ricinus* tick hosts and assess *Borrelia* infection in the United Kingdom H. Broadhurst (Salford/UK)
09:25	PP 101 New insights on *Babesia divergens* and *Ixodes ricinus* interaction: piroplasm expression of apical complex proteins during salivary gland invasion and development V. Risco-Castillo (Maisons-Alfort/FR)
09:30	PP 072 The understudied role of winter ticks (*Haemaphysalis inermis*) in pathogen transmission É. Szabó (Budapest/HU)
09:35	PP 102 In silico structural biology to understand tick-microbe interactions: the case study of *Midichloria mitochondrii* D. Sassera (Pavia/IT)

09:40	PP 092 Temporal changes in tick-borne pathogens prevalence observed in questing *Ixodes ricinus* (Linnaeus, 1758) across different habitat types in north eastern Italian Alps G. Ferrari (San Michele all'Adige/IT)
09:45	PP 093 Environmental influences on tickborne pathogen prevalence and *Peromyscus leucopus* population size in Pennsylvania, USA M. Rowley (East Stroudsburg, PA/US)
09:50	PP 065 Tick-borne pathogen prevalence and density of blacklegged ticks in four Pennsylvania counties N. Chinnici (East Stroudsburg, PA/US)
09:55	PP 066 Ticks in the city versus in forests – mobile app shows risk of infection K. Kybicová (Prague/CZ)
10:00	PP 095 Prevalence and predictors of tick-borne pathogens in deer communities M. Holding (Porton Down/UK)
10:05	PP 103 *Borrelia lusitaniae* and *Borrelia burgdorferi* sensu stricto are preferentially maintained by sand lizards (*Lacerta agilis*) and their immature *Ixodes ricinus* ticks, in western Poland J. Michalik (Poznań/PL)
10:10	PP 069 Predator-prey dynamics and tick-borne disease risk N. De Pelsmaeker (Białowieża/PL)
10:20–11:50	Poster session II and coffee break | Belvedere

11:50–12:20	Keynote V
Room	Goethesaal
Chairs	H. Sprong (Bilthoven/NL), A. Springer (Hannover/DE)
11:50	OP 057 The concept of natural transmission of TBE virus revisited G. Dobler (Munich/DE)
12:15	Discussion
12:20–13:10	Session XV | Tick-host interactions and pathogen transmission dynamics
Room	Goethesaal
12:20	OP 058 Overwintering, trans-stadial transmission and long persistence of the European and Siberian tick-borne encephalitis virus strains in *Ixodes persulcatus*, *Ixodes ricinus* ticks and their hybrids O. Belova (Moscow/RU)
12:35	OP 059 The role of ungulates in tick-borne pathogen epidemiology – bloodmeal duration affects *Borrelia* spp. and *Anaplasma phagocytophilum* infection status and pathogen load in *Ixodes ricinus* A. Springer (Hannover/DE)
12:45	OP 060 Immune response to alpha-Gal: Disease biomarkers and control of tick infestations and infectious diseases J. de la Fuente (Ciudad Real/ES)
13:00	Discussion

13:10–14:30	Session XVI | Insights into tick physiology, immunomodulation and microbe interactions
Room	Goethesaal
Chairs	P. Kopacek (České Budějovice/CZ), L. Simo (Maisons-Alfort/FR)
13:10	OP 061 Transcriptomic and proteomic insights into the longitudinal dynamics of midgut physiology in *Ixodes* sp. females P. Kopacek (České Budějovice/CZ)
13:25	OP 062 Insight into the tick neurosecretory system L. Simo (Maisons-Alfort/FR)
13:40	OP 063 Functional Insights into tick-microbe interactions J. Perner (České Budějovice/CZ)
13:50	Discussion
14:00	OP 064 The tick-pathogen anti-complement evolution dictates host skin immunomodulation for multimodal microbial transmission Y.-P. Lin (North Grafton, MA/US)
14:15	OP 065 A dynamic histamine continuum shapes the tick-host interaction L. Robbertse (České Budějovice/CZ)
14:25	Discussion
14:30	Awards and Closing ceremony

12:10–13:10 Poster session I
Room Belvedere

Borrelia

PP 001

Structural and functional characterisation of the β-barrel assembly machinery complex from the Lyme disease causing agent *Borrelia burgdorferi*
K. Brangulis (Riga/LV)

PP 002

Evolution of the *vls* antigenic variation system in Lyme *Borrelia* genomes
A. Collado (New York, NY/US)

PP 003

Optimising Transformation Efficiency in *Borrelia* using in vitro methylated plasmids to bypass the restriction-modification system
M. Ruivo (Vienna/AT)

PP 004

Adhesion of *Borrelia* and other human pathogenic bacteria to endothelial cells is facilitated by fibronectin interaction
D. J. Vaca (Frankfurt a. M./DE)

PP 005

Early pathogenic mechanisms of Lyme disease: Volume electron microscopy insights into *Borrelia burgdorferi* barrier penetration and spread
M. Vancová (České Budějovice/CZ)

PP 006

Retention of lp56 in *Borrelia burgdorferi* transposon mutants and investigating the importance of the bactofilin *fapA* (BB0267) for infection
R. Rego (České Budějovice/CZ)

Citizen Science

PP 007

Bites of knowledge – citizen science focused on schools to increase awareness and knowledge on ticks
R. Rodríguez-Pastor, A. Estrada-Peña (Zaragoza/ES)

PP 008

Ticks and tick-borne pathogens in Austria 2024
A.-M. Schötta (Vienna/AT)

PP 009
Community-Driven Tick Collection – a study on the distribution of different tick species from multiple domestic hosts in Mecklenburg-Western Pomerania and Schleswig-Holstein
S. Fischer (Greifswald/DE)

PP 010
New findings of *Hyalomma marginatum* and *Ixodes frontalis* in Slovakia
M. Kazimírová (Bratislava/SK)

PP 011
Emergence of *Hyalomma* ticks in Poland: the contribution of citizen science to tick surveillance
W. Romanek (Warsaw/PL)

Tick genomics

PP 012
The Tick Cell Biobank – application of tick cell lines in tick mitogenomic studies
C. Hartley (Liverpool/UK)

PP 013
The current state of tick genomics
I. Ronai (Cambridge, MA/US)

PP 014
EZeSA: Microbiome of ticks in Saxony-Anhalt, Germany
A. Katsounas (Magdeburg/DE)

Tick microbiome

PP 015
Update on tick-borne pathogens and new insights into the microbiota of hard ticks in Portugal
L. Moerbeck (Lisbon/PT)

PP 016
Comparative bacterial communities and population genetics between bisexual and parthenogenetic *Haemaphysalis longicornis* in South Korea
J. Kim (Sangju-si/KR)

Tick physiology

PP 017
Identification of ion transport peptide-like (ITPL) signalling in the tick *Ixodes ricinus*
V. Klöcklerová (Bratislava/SK)

PP 018
Enzymes beyond tick midgut hemolysis
T. Kozelková (České Budějovice/CZ)

PP 019
Microscopic anatomy of the midgut of *Ixodes ricinus* nymphs in different feeding stages
V. Urbanova (České Budějovice/CZ)

PP 020
Molecular screening of the physiological roles of neuropeptides and GPCRs in attachment and salivary secretion from *Haemaphysalis longicornis*
D. Kim (Sangju-si/KR)

PP 021
Optimization of a novel artificial feeding system for the lone star tick, *Amblyomma americanum*
M. Burch (Galveston/US)

Tick-borne viruses

PP 022
Results from eight years of surveillance of ticks and tick-borne encephalitis virus in southern Norway
A. P. Cotes Perdomo (Oslo/NO)

PP 023
Establishing multicellular blood-brain barrier infection models for antiviral drug discovery
M. Zuniga (Oberschleissheim/DE)

PP 024
Prevalence and risk factors of tick-borne encephalitis virus in dogs in Lithuania
J. Radzijevskaja (Kaunas/LT)

PP 025
Studies on the co-localisation of TBE-virus and *Borrelia* species at selected TBEV-foci in Baden-Wuerttemberg
R. Vogt (Stuttgart/DE)

PP 026

Tick-borne encephalitis virus NS1 disrupts the integrity of endothelial monolayer
M. Čížková (České Budějovice/CZ)

PP 027

Characterisation of tick-borne encephalitis virus from *Ixodes ricinus* ticks from eastern German low mountain ranges
M. Pfeffer (Leipzig/DE)

PP 028

Wild rodents as sentinel hosts for tick-borne encephalitis virus – a study of TBEV prevalence in TBEV foci in Lithuania
A. Pautienius (Kaunas/LT)

PP 029

Quasispecies in tick-borne encephalitis virus – utilising long-read next generation sequencing to determine population diversity and genomic plasticity in tick-borne flaviviruses
L. Tardy (České Budějovice/CZ)

PP 030

Characterisation of viral-derived DNA (vDNA) forms in *Hyalomma lusitanicum* tick cell line in response to Hazara virus infection
E. Dias (Lisbon/PT)

PP 031

Tick-species and TBEV surveillance in Bronnoy, Northern Norway
A. Saiyara (Oslo/NO)

PP 032

Epidemiology of Crimean-Congo hemorrhagic fever virus in Tunisia, North Africa
E. Zhioua (Tunis/TN)

PP 033

Identifying primary and alternative tick vectors for Crimean-Congo hemorrhagic fever virus: The importance of defining vectorial capacity of ticks for human risk
D. Bente (Galveston/US)

Ticks and tick-borne pathogens of veterinary importance

PP 034
Tick-borne pathogens in pet and hunting dogs in Kaunas, Lithuania
K. Jankauskaitė (Kaunas/LT)

PP 036
Two complete genomes of *Babesia divergens* derived from infected cattle in Great Britain
N. Johnson (Addlestone/UK)

PP 037
Retrospective hematological analysis of *Babesia canis* infections in dogs in Germany (2018-2024)
I. Schäfer (Bad Kissingen/DE)

PP 038
Low serological reactivity to *Rickettsia* in dogs from the surroundings of an atlantic rainforest reserve – the influence of large predators?
M. P. J. Szabó (Uberlândia/BR)

PP 039
Introduction of exotic ticks (*Amblyomma geoemydae*) in Sweden along with illegal imported turtles (G*eoemyda spengleri*) from Thailand
L. Chitimia-Dobler (Munich/DE)

PP 040
New insights into occurrence of tick-borne pathogens in Zambia
A. Schulz (Greifswald/DE)

PP 041
Investigation of a piroplasm outbreak in a herd of horses in Southern Germany
R. Vogt (Stuttgart/DE)

PP 042
Molecular detection *Theileria annulata* in cattle from South Kazakhstan
K. Mukanov (Astana/KZ)

PP 043
Distribution of *Babesia* spp. in cattle across the Southern and Central regions of Kazakhstan
N. Tursunbay (Astana/KZ)

PP 044
Ticks on cats and dogs in Northern Cyprus
A. Taylan-Özkan (Ankara/TR), K. Mumcuoglu (Jerusalem/IL)

PP 045
Is anaplasmosis endemic in smallholder cattle and buffaloes in Pakistan – evidence from molecular phylogenetic analysis
A. Ghafar (Melbourne/AU)

PP 046
Presence of *Anaplasma phagocytophilum* in lambs at post mortem is associated with an increased likelihood of co-infection with Pasteurellaceae bacteria and a diagnosis of pneumonia
J. Gilmore (Elphin/IE)

10:20–11:50 Poster session II
Room Belvedere

Diagnostics

PP 047
Recombinant Spm2 protein for serological diagnosis of cattle theileriosis
A. Ryskeldina (Astana/KZ)

PP 048
Regional differences in tick-borne encephalitis diagnostics across Germany – a three-year multicenter study
J. Leifheit (Berlin/DE)

PP 049
Discrepancies in widely used serological methods to detect *Borrelia* infections may cause missed Lyme diagnoses
E. Volokhina, A. Garritsen (Oss/NL)

PP 050
Improvement of the serodiagnosis of tick- and louse-borne relapsing fever
F. Reyer (Frankfurt a. M./DE)

PP 051
A new method for rapid and accurate detection of *Borreliaceae* DNA based on *flaB* gene sequences
A. Taylor (Poznań/PL)

PP 053
Comparison of the diagnostic performance of PCR kits for detecting tick-borne pathogens‘ in tick specimens
S. Sadangi (Bonn/DE)

PP 054
Detection and isolation of *Francisella tularensis* using recombinant reporter fusion proteins
J. K. Janssen (Munich/DE)

Field ecology

PP 055
Innovative machine learning algorithms to understand the driving forces for tick abundance across Europe
S. Lansdell (London/UK)

PP 056
The Zanzemap project – artificial intelligence models to forecast vector dynamics in Northern Italy
G. Marini (San Michele all'Adige/IT)

PP 057
First insights into pathogen presence and diversity in the Luxembourg's non-native *Dermacentor reticulatus* tick population
P. Reteng (Esch-sur-Alzette/LU)

PP 058
Ixodes ricinus ticks survive flooding
J. Rapp (Hannover/DE)

PP 059
The survivability of *Rhipicephalus sanguineus* s.s. and *Rh. linnaei* in Germany – a controlled outdoor study
K. Fachet-Lehmann (Stuttgart/DE)

PP 060
Tick distribution and phenology across altitudinal gradients in the central Pyrenees, Spain: preliminary results
S. Soares (Zaragoza/ES)

PP 061
Ticks and tick-borne pathogens in the city park of Greifswald, an urban area of Mecklenburg-Western Pomerania, in 2022
S. Fischer (Greifswald/DE)

PP 062
Tick abundance and tick-borne pathogen prevalences in different ecosystems in the Czech Republic in 2024
E. Richtrová (Prague/CZ)

PP 063

Utilising retrotransposon blood meal analysis (Rt-BMA) to identify *Ixodes ricinus* tick hosts and assess *Borrelia* infection in the United Kingdom
H. Broadhurst (Salford/UK)

PP 064

A resource-based habitat concept for ticks with a focus on forest as land systems
S. O. Vanwambeke, L. Beautrix (Louvain-la-Neuve/BE)

PP 065

Tick-borne pathogen prevalence and density of blacklegged ticks in four Pennsylvania counties
N. Chinnici (East Stroudsburg, PA/US)

PP 066

Ticks in the city versus in forests – mobile app shows risk of infection
K. Kybicová (Prague/CZ)

PP 067

Updated distribution of *Dermacentor* ticks in Austria
M. Wijnveld (Vienna/AT)

PP 068

Ticks and the city: investigating ticks and tick-borne diseases in urban and peri-urban environments
M. Burdukiewicz (Białystok/PL)

PP 069

Predator-prey dynamics and tick-borne disease risk
N. De Pelsmaeker (Białowieża/PL)

PP 107

Molecular survey of tick-borne pathogens in dogs and their ticks in France
Ambre Sibari (Libourne/FR)

Lesser known ticks

PP 070

Unexplored ticks – studying lesser-known species as the key to understanding global pathogen vectors
D. Wężyk (Warsaw/PL)

PP 071

Ixodes laguri, a grassland and steppe tick that parasitizes critically endangered rodents
F. Rubel (Vienna/AT)

PP 072
The understudied role of winter ticks (*Haemaphysalis inermis*) in pathogen transmission
É. Szabó (Budapest/HU)

PP 073
First report of *Ixodes inopinatus* from the Akrotiri wetland in Cyprus – Insights from tick surveillance and pathogen analysis
A. Saratsis (Thermi/GR)

Public Health

PP 074
Using meta-analysis to estimate the incidence of the clinical manifestations of Lyme borreliosis in Denmark
G. Brestrich (Berlin/DE)

PP 075
Incidence and manifestations of Lyme borreliosis in high-endemic sites in Germany – the Burden of Lyme Disease (BOLD) study
C. Malerczyk (Berlin/DE)

PP 076
Beware of hitchhiking ticks? Clarifying the variable roles of bird species in tick movement along migratory routes
R. E. Rollins (Wilhelmshaven/DE)

PP 077
Borrelia burgdorferi s.l. in ticks from Lyme neuroborreliosis-risk areas in Denmark
N. Król (Leipzig/DE)

PP 078
Prevalence of *Borrelia burgdorferi* sensu lato infections in Switzerland, 2022
R. Lienhard (La Chaux-de-Fonds/CH)

PP 079
Diversity of ticks on trails in asturias (northwest Spain) – big differences depending on the study area
A. Espí Felgueroso (La Olla (Gijón)/ES)

PP 080
Burden of Lyme disease (BOLD) study – health-related quality of life among clinically diagnosed subjects with Lyme borreliosis by manifestation and compared with controls in six European countries
H. Yu (Collegeville,PA/US)

PP 081
Ticks and tick-borne pathogens in urban areas in Lithuania
J. Radzijevskaja (Kaunas/LT)

PP 082
Molecular detection of *Borrelia* spp. in red squirrels (*Sciurus vulgaris*) and their ticks
U. Medikaitė (Kaunas/LT)

PP 083
The raccoon (*Procyon lotor*) as an emerging neozoon and potential reservoir for tick-borne pathogens in Germany
L. M. I. Maas (Leipzig/DE)

PP 084
Nationwide survey of *Borrelia* prevalence and species distribution in *Ixodes ricinus* ticks in Germany
J. R. Raasch (Hannover/DE)

PP 085
Vector-free transmission of Lyme disease spirochetes
N. Rudenko (České Budějovice/CZ)

PP 086
Concurrent infection of the human brain with multiple species of Lyme disease spirochetes
N. Rudenko (České Budějovice/CZ)

PP 087
Health claims data reveal high incidence of tick-borne encephalitis in Germany
G. Levy (Berlin/DE)

PP 088
An update on the estimate of Lyme disease incidence in England
J. Olver (Salisbury/UK)

PP 089
Borrelia burgdorferi s.l. abundance and genetic diversity between urban greenspaces and surrounding hinterland across the UK
G. Plahe (Salford/UK)

PP 090
First findings of *Babesia* spp. and *Rickettsia* spp. in bats (Chiroptera) in Slovakia
M. Kazimírová (Bratislava/SK)

PP 091

Ectoparasites associated with rodents in different regions of Iran
N. Moradpoor Sheykhkanloo (Konesin/CZ)

PP 092

Temporal changes in tick-borne pathogens prevalence observed in questing *Ixodes ricinus* (Linnaeus, 1758) across different habitat types in north eastern Italian Alps
G. Ferrari (San Michele all'Adige/IT)

PP 093

Environmental influences on tickborne pathogen prevalence and *Peromyscus leucopus* population size in Pennsylvania, USA
M. Rowley (East Stroudsburg, PA/US)

PP 094

Knowledge, attitudes, and practices regarding tick bites and tick-borne diseases – insights from high-risk groups at an *Ixodes ricinus* hotspot in Greece
A. Saratsis (Thermi/GR)

PP 095

Prevalence and predictors of tick-borne pathogens in deer communities
M. Holding (Porton Down/UK)

PP 096

Wild carnivores are potential reservoir hosts for *Anaplasma phagocytophilum* strains pathogenic for humans
F. D. von Loewenich (Mainz/DE)

PP 097

Genetic diversity of tick-borne zoonotic pathogens in ixodid ticks collected from small ruminants in Northern Pakistan
J. Zeb (Hong Kong/HK)

PP 106

First detection of Borreliella spirochetes from the Lyme borreliosis group in hematophagous mites of the genus Spinturnix (Acari: Mesostigmata) parasitizing Myotis myotis and M. daubentoni bats (Vespertilionidae) in Poland
B. Sikora (Pozna□ /PL)

Tick control

PP 098

Comparative biochemical characterisation and inhibitory profiling of cattle tick, human, bovine and mosquito Flavin Adenine Dinucleotide sub-domains
R. Finn (Newcastle Upon Tyne/UK)

Tick-host pathogen interactions

PP 099

Role of complement on host adaptation of *Borrelia garinii* in seabirds – insights into the terrestrial and marine cycle transmission
P. Kraiczy (Frankfurt a. M./DE)

PP 100

Are rodents key hosts for *Ixodes ricinus* larvae? Insights from blood meal analysis in German ticks
A. Springer (Hannover/DE)

PP 101

New insights on *Babesia divergens* and *Ixodes ricinus* interaction: piroplasm expression of apical complex proteins during salivary gland invasion and development
V. Risco-Castillo (Maisons-Alfort/FR)

PP 102

In silico structural biology to understand tick-microbe interactions: the case study of *Midichloria mitochondrii*
D. Sassera (Pavia/IT)

PP 103

Borrelia lusitaniae and *Borrelia burgdorferi* sensu stricto are preferentially maintained by sand lizards (*Lacerta agilis*) and their immature *Ixodes ricinus* ticks, in western Poland
J. Michalik (Poznań/PL)

PP 104

Agent based modeling of a vector-host-pathogen system elucidates the effects of immune selection and host specialization on antigen evolution and strain structure in *Borrelia* populations
B. Ely (New York, NY/US)

PP 105

Unravelling the versatility of tick antimicrobial peptides: From immunity to pathogen control and vector competence
M. Tonk-Rügen (Gießen/DE)

We thank all industrial partners for supporting ISTTBD-XVI 2025.

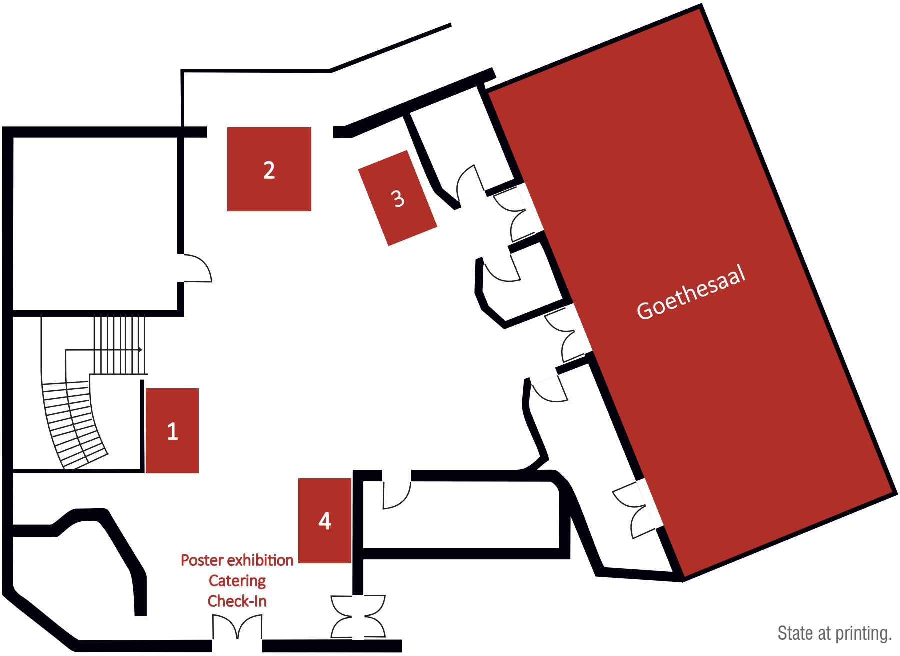
Main sponsors
Pfizer Pharma GmbH

Bavarian Nordic

Elanco Deutschland GmbH

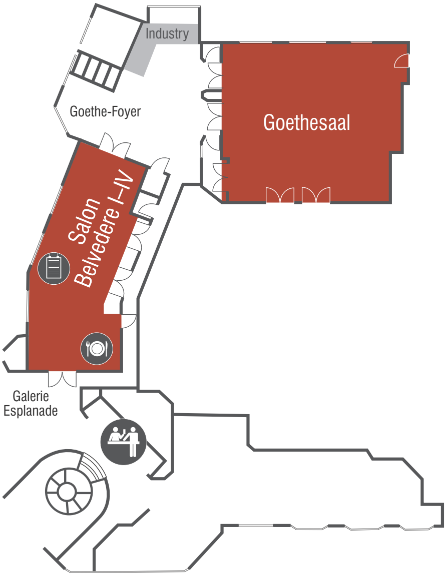
Exhibitors	Booth no.
Autoimmun Diagnostika GmbH	4
Bavarian Nordic	1
Elsevier B.V.	3
Pfizer Pharma GmbH	2

Further sponsors
tick-radar GmbH

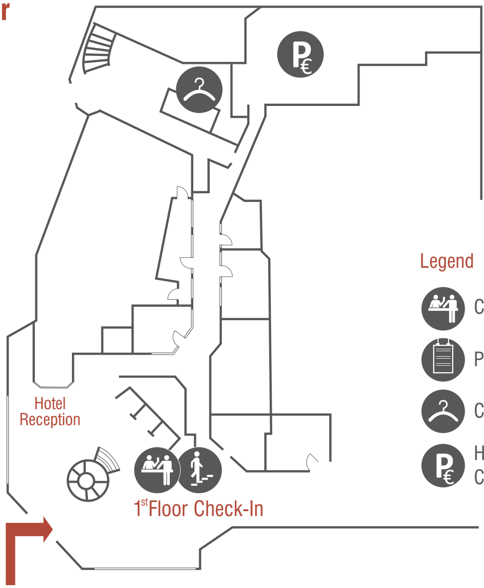


State at printing.

1st Floor



Ground Floor



Legend

- Check-In ISTTBD-Conference
- Poster Exhibition
- Cloakroom
- Hotel Underground Car Park – with costs

State at printing.

Social and cultural programme

Welcome reception I Wednesday, 26 March

The organisers welcome all participants of the conference in the room “Belvedere” and in the Foyer. Meet colleagues and other participants while enjoying snacks and beverages.

Time	19:30
Place	Belvedere & Foyer

Social evening I Thursday, 27 March

We invite you to join a memorable evening at the “Villa Haar” in Weimar. Celebrate the return of the 16th International Symposium on Ticks and Tick-borne diseases together with your colleagues and friends. Enjoy the exclusive setting of the historic Italian style villa built in 1885.

Time	20:00
Place	Villa Haar I Dichterweg 2A | 99425 Weimar

The “Villa Haar” is only a 15 minutes stroll away from the Leonardo Hotel Weimar. The walk will take you through the beautiful Ilm Park.

© drizzlingstarsstudio I AdobeStock / Harald Wenzel-Orf

General terms and conditions

Please find our general terms and conditions at www.ittbd-symposium.com.

Conference Venue

Leonardo Hotel Weimar I Belvederer Allee 25 I 99425 Weimar, Germany

Parking

The location offers the following parking possibilities:
On-site outside parking 10 EUR per day and the Hotel car park is 12 EUR per day.

Public transportation from the main station to the venue

Weimar Main Train Station (Hauptbahnhof/Hbf) is about 4 km far away from the Bus stop "Falkenburg" (50 m walk to the Leonardo Hotel). Take Line 1, Ehringsdorf, Kippergasse. The bus trip takes around 14 to 20 min.

Certification

The symposium is accredited by the "ATF" (Akademie für ärztliche Fortbildung der Bundestierärztekammer e.V.) with 19 education hours.

Certificate of attendance

Certificates of attendance will be sent out to the participants afterwards via E-Mail.

Name badge

Please wear your name badge during all conference events, including the networking activities. Admission to scientific sessions and to the industrial exhibition is restricted to participants wearing their badge. Participants will receive their name badge at the check-in desk.

Check-In

The check-in desk is going to be located at first floor in the Goethe Foyer.

Cloakroom

The coat and luggage room at the conference can be found on the ground floor, past the hotel reception in front of the banquet foyer. The fee is 1 EUR per garment or luggage.

WIFI access

WIFI is available free of charge throughout the whole conference area. Please ask at the check-in desk for the login data.

Conference language

The official conference language is English.

Publication of abstracts

All abstracts will be published in the main programme book.

Awards

A jury will select the three best presentations given during the Sinnecker-Kunz session for early-career researchers. A second jury will select the two best posters.

Catering

The catering stations are located in the Belvedere room and foyer. During the coffee breaks, coffee and tea are offered free of charge at the catering stations. The lunch snack is included in your conference fee. During the Welcome Reception catering will be offered free of charge.

Presentation upload

The media check-in for uploading your presentation is located in the back of the plenary Goethesaal (please follow the sign posting on site). For submission, please use a USB flash drive. Professional staff and equipment will be available for you to arrange and preview your presentation.

Time allocation

Please prepare your presentation for the allotted amount of time. Chairs and moderators may interrupt should you overrun your time limit.

Poster sessions

Two dedicated poster sessions will be held during the ISTTBD-XVI:

- Thursday, 27 March 2025 12:20-13:10
- Friday, 28 March 2025 10:20-11:50

Presenting authors are requested to be available during their poster sessions. Online poster presenters can be contacted over the chat online, to discuss the poster.

All poster boards will be labelled with a poster number. You can find your poster number in the programme book.

The size of your poster has to be in accordance with DIN format A0 (84.1 cm width x 118.9 cm height) and should not be laminated.

Pins will be provided on your poster board. Please do not use any other type of pins than those provided.

Posters have to be attached by Wednesday, 26 March from 13:00 and have to be removed by 16:00 on Friday, 28 March.

Sinnecker-Kunz Award

The Sinnecker-Kunz Award for early-career researchers (ECRs) will be presented during the 16th International Symposium on Ticks and Tick-borne Diseases (ISTTBD-XVI) in 2025.

The aim of the award is to acknowledge distinguished achievements by ECRs. The award is named in honour of the virologist Professor Herbert Sinnecker, who was the first to find tick-borne encephalitis (TBE) virus in Germany and the virologist Christian Kunz, who developed the first European TBE vaccine. This is the seventh time that the award will be presented.

Please note: The programme takes place according to the time zone CEST.

Registration

Please register online at www.ittbd-symposium.com. There you can find all information on registration fees, payment and confirmation of payment as well as the general terms and conditions.

Log-in on the digital congress page

You can access the digital congress page via www.ittbd-symposium-digital.com. On the starting page you can register for the congress at any time by clicking on “New registration”.

If you have already registered, you can reach the login portal of the congress by clicking “Login”. Now you can enter the world of the digital ISTTBD-XVI.

Technical requirements

To ensure the best possible transmission quality and stability, the use of a direct LAN connection (as opposed to WiFi) is recommended.

Before the meeting, make sure that sound and video quality of your computer/notebook is good. Make sure that you are using the latest version of your browser. Using Internet Explorer as your browser is not recommended. Also make sure that you do not have too many tabs open in your browser.

To participate in the scientific programme

Please check if your browser and operating system support the playback of Vimeo: https://vimeo.zendesk.com/hc/en-us/articles/360001624108-System-Requirements-for-viewingbrowsing-and-apps.

Procedure of Keynote lectures, scientific sessions and poster sessions

Talks given online for keynote lectures, scientific sessions and posters will be held live via screensharing. In the keynote lectures and scientific sessions, the session chairs will introduce the speakers and talk starts afterwards. The chairs and speakers are connected live. Participants can ask questions via chat, the questions are viewed by the chairs and passed on to the speakers, who answer them live.

On Demand – congress material on demand

Posters will be available online for individual review until 30 April 2025.

A
Abiun-Denis, L. 9, 207

B
Beautrix, L. 30, 161
Belova, O. 20, 72, 203
Bente, D. 12, 25, 95, 107
Birtles, R. 16, 138, 160, 186
Boulanger, N. 8, 56
Brangulis, K. 22, 75
Brestrich, G. 31, 171, 175
Broadhurst, H. 18, 30, 160
Burch, M. 24, 95, 107
Burdukiewicz, M. 30, 165

C
Cabezas Cruz, A. 10, 61, 67
Cadar, D. 88
Chinnici, N. 19, 30, 50, 162, 190
Chitimia-Dobler, L. 14, 26, 51, 10, 112, 113, 125, 144, 167, 170
Choi, O. N. 7, 49, 142
Čížková, M. 12, 25, 70, 100
Collado, A. 12, 22, 76
Cotes Perdomo, A. P. 12, 24, 96, 105
Cutler, S. 18, 152

D
Dagostin, F. 11, 52, 73, 153, 189
Dautel, H. 14, 15, 16, 62, 132
de la Fuente, J. 11, 20, 205
De Pelsmaeker, N. 19, 30, 166
Deshpande, S. 14, 128
Dias, E. 25, 104
Dirnagl, U. 11, 69
Dobler, G. 20, 65, 74, 101, 144, 184

E
Elati, K. 9, 62, 141
Ely, B. 34, 201
Espí Felgueroso, A. 31, 176
Estrada-Peña, A. 15, 18, 22, 81, 130, 133, 134
Fachet-Lehmann, K. 18, 29, 74, 99, 114, 156
Ferrari, G. 19, 33, 52, 189
Fingerle, V. 12, 46, 47, 48, 148, 196, 204
Finn, R. 34, 195
Fischer, S. 23, 29, 83, 158
Foley-Eby, A. 9, 60

G
Garritsen, A. 28, 147
Geginat, G. 88
Ghafar, A. 27, 61, 118
Gilmore, J. 27, 119

H
Hartley, C. 23, 68, 86
Hepner, S. 7, 46, 47, 48, 196
Holding, M. 19, 33, 64, 106, 192
Hönig, V. 8, 53, 59, 70, 103, 159, 163

J
Jacobs, E. 16, 136
Jankauskaitė, K. 26, 108
Janssen, J. K. 28, 151
Jlassi, O. 14, 106, 126
Johnson, N. 26

K
Kahl, O. 11, 181, 197
Kamiš, J. 9, 53, 59, 103, 159, 163
Katsounas, A. 88
Kazimírová, M. 8, 23, 32, 54, 68, 84, 187
Kelava, S. 15, 16, 17, 129
Kelly, P. 15, 133, 134
Khoo, J. J. 10, 68, 86, 138
Kim, J. 23, 90, 94
Kim, D. 24, 90, 94
Klafke, G. 15, 16, 17, 131, 135
Klöcklerová, V. 14, 24, 91
Kopacek, P. 21, 92, 93, 206, 208
Kotál, J. 11, 71
Kozelková, T. 14, 24, 92, 93
Kraiczy, P. 7, 34, 47, 78, 148, 196, 209
Krawczyk, A. I. 10, 64, 65, 154
Król, N. 18, 31, 174, 180
Kybicová, K. 19, 30, 53, 59, 159, 163

L
Lansdell, S. 18, 29, 152
Lao-Gonzalez, T. 9, 58
Leifheit, J. 28, 146
Lesiczka, P. 10, 65
Levy, G. 32, 184
Lienhard, R. 31, 175
Lin, Y.-P. 21
Lindau, A. 11, 74, 99, 114, 156
Luehken, R. 88

M
Maas, L. M. I. 32, 101, 180
Malerczyk, C. 31, 146, 172, 184
Mans, B. J. 14, 46, 124, 125
Margos, G. 7, 13, 46, 47, 48, 196
Marini, G. 29, 49, 52, 73, 142, 153, 189
Medikaitė, U. 32, 179
Melis, S. 10, 66, 199
Michalik, J. 19, 34, 149, 200
Moerbeck, L. 12, 23, 89
Moniuszko, A. 13, 120, 177
Moradpoor Sheykhkanloo, N. 33, 188
Mukanov, K. 26, 115
Mumcuoglu, K. 27, 117
Muñoz Leal, S. A. 10
Mustafa, B. E. 9, 61

N
Nadolny, R. 17, 143
Nath, T. C. 109
Nigrovic, L. 13, 121
Nock, A. 16, 139

O
Olver, J. 32, 185

P
Palus, M. 11, 53, 59, 70, 100, 103
Pautienius, A. 25, 98, 102
Perner, J. 14, 15, 16, 21, 57, 127, 199, 208, 210
Pfeffer, M. 9, 25, 101, 144, 174, 180, 181
Pinarello Kleiber, V. 16, 137
Plahe, G. 32, 186
Polienko, A. 11, 72, 203

R
Raasch, J. R. 32, 181
Radzijevskaja, J. 24, 32, 98, 102, 108, 178, 179
Rakotobe, S. 14, 57, 127, 198, 207
Rapp, J. 8, 29, 55, 155
Reck, J. 16, 131, 135
Rego, R. 22, 79, 80
Reteng, P. 29, 154
Reyer, F. 18, 28, 148
Richtrová, E. 29, 53, 159, 163
Risco-Castillo, V. 18, 34, 198
Rizzoli, A. 11, 52, 73, 153, 189
Robbertse, L. 21, 208, 210
Rodríguez-Pastor, R. 22, 81, 157
Rollins, R. E. 8, 31, 51, 170, 173, 196
Romanek, W. 23, 85, 167
Ronai, I. 13, 23, 87, 123
Rounsville Jr., T. F. 17, 49, 142
Rowley, M. 19, 33, 50, 162, 190
Rubel, F. 30, 168
Rudenko, N. 32, 182, 183
Ruivo, M. 22, 77, 164, 169, 187
Ryskeldina, A. 28, 116, 145

S
Sadangi, S. 28, 150
Saiyara, A. 25, 105
Saratsis, A. 31, 33, 170, 191
Sassera, D. 18, 34, 66, 199, 208
Schäfer, I. 26, 110
Schmidt-Chanasit, J. 88
Schötta, A.-M. 22
Schulz, A. 26, 113
Schwartz, S. 8, 50, 162, 190
Simo, L. 21, 65, 127, 198, 207, 210
Soares, S. 29, 157
Springer, A. 20, 34, 55, 155, 181, 197, 204
Sprong, H. 20, 53, 64, 65, 66, 106, 192
Strle, K. 7, 45
Strube, C. 9, 55, 110, 155, 181, 197, 204
Szabó, M. P. J. 26, 111
Szabó, É. 18, 31, 169

T
Tagliapietra, V. 8, 52, 73, 189
Tajeri, S. 17, 141
Tardy, L. 12, 25, 59, 103
Taylan-Özkan, A. 27
Taylor, A. 28, 149
Tonk-Rügen, M. 34, 202
Tursunbay, N. 26, 116, 145

U
Urbanova, V. 24, 93, 208

V
Vaca, D. J. 22, 78
Vancová, M. 22, 57, 79, 80
Vanwambeke, S. O. 30, 161
Vlot, M. 16, 140
Vogt, R. 12, 24, 26, 99, 114
Volokhina, E. 28, 147
von Loewenich, F. D. 33, 193

W
Wężyk, D. 13, 30, 122, 167
Wijnveld, M. 30, 77, 164, 169, 187

Y
Yu, H. 31, 177

Z
Zeb, J. 33, 194
Zhioua, E. 25, 106
Zintl, A. 12, 119
Zuniga, M. 12, 24, 97

ISTTBD-XVI

16th International Symposium on Ticks and Tick-borne Diseases

ABSTRACTS

Order analogous to how the abstracts appear in the programme

OP 001

Immune response in the pathogenesis of Lyme disease

K. Strle[1], M. A. Williams[1], S. A. Hernandez[1], I. Morban Mora[1], S. L. Arvikar[2], F. Strle[3], C. Wells[1]
A. C. Steere[2], L. Hu[1], T. Petnicki-Ocwieja[1]
[1]Tufts University School of Medicine, Molecular Biology and Microbiology, Boston/MA, USA
[2]Massachusetts General Hospital, Rheumatology, Boston/MA, USA
[3]University Medical Centre Ljubljana, Department of Infectious Diseases, Ljubljana, Slovenia

Lyme disease, presents with a range of manifestations which vary in severity and duration, including chronic pain, fatigue or neurocognitive symptoms after antibiotic therapy for the infection (PTLDS). A central question is why some patients have mild self-limiting symptoms whereas others develop severe and persistent disease. Emerging evidence points to inappropriate immune responses, which are shaped at least in part by host genetic variation, as a possible cause of clinical heterogeneity.

Using a translational approach in human immunology, we found that patients with PTLDS have significantly elevated levels of IL-23 and IFNα in serum compared to patients whose symptoms resolve. Moreover, ex vivo cell culture experiments demonstrate that cells from PTLDS patients have excessive inflammatory responses to *B. burgdorferi*, implying that these patients are genetically prewired for excessive immune responses. Using targeted and discovery approaches we have identified several genetic risk factors in Lyme disease. In particular, we found that a mutation in the TLR1 gene, a key sensor for *Borrelia*, is associated with excessive inflammation and severe disease in patients. This enhanced response to *B. burgdorferi*, appears to be due to a lack of appropriate regulation. Repeat stimulation with *B. burgdorferi*, which mimics tolerogenic conditions during infection, failed to induce innate immune tolerance, resulting in seemingly unabated inflammatory responses similar to those observed in patients with post-antibiotic sequelae.

Collectively, these findings suggest that excessive inflammation and more severe disease in may be due to inappropriate regulation and inability of the host to induce immune tolerance to infection. These results help explain how early events in the infection may contribute to sustained aberrant immune activation observed in patients with post-Lyme sequelae and solidify the role of host genetic variation in shaping these outcomes.

OP 002

The completed genome of *Borrelia tillae* – a relapsing fever *Borrelia* species from South Africa

V. Fingerle[1], S. Hepner[1], D. Velasquez[1], K. Kuleshov[2], F. Schedel[3], S. Stockmeier[1], C. Hartberger[1]
T. Schwan[4], B. J. Mans[5], R. Pienaar[5], G. Margos[1]
[1]Bavarian Health and Food Safety Authority, National Reference Center for Borrelia, Oberschleissheim, Germany
[2]Central Research Institute of Epidemiology, Moscow, Russian Federation
[3]Bavarian Health and Food Safety Authority, Oberschleissheim, Germany
[4]National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Laboratory of Bacteriology, Hamilton, MT, United States[5]Argricultural Research Council - Onderstepoort Veterinary Research, Epidemiology, Parasites and Vectors, Onderstepoort, South Africa

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 a *Borrelia* species found in *O. zumpti* and named it *B. tillae*. The *Borrelia* species showed phenotypical and biochemical differences compared to *B. duttonii*. It was infectious in laboratory mice and multimammate rats. An attempt to infect *O. moubata* Murray 1877 proved unsuccessful but it was successfully isolated from *O. zumpti*.

DNA from the cultured *B. tillae* isolate Krampitz yielded sequences for 16S rRNA, *flaB* and multilocus sequence typing (MLST) loci and phylogenetic trees. 16S rRNA sequences were also obtained from an *O. zumpti* colony. A whole genome assembly was constructed using sequences generated with Illumina, Oxford Nanopore and Pacific Biosciences technologies.

No close matches to 16S rRNA, *flaB* or MLST loci were found in BLAST searches. Genetic distance analyses confirmed the species status of *B. tillae*. Borrelial 16S rRNA sequences from an *O. zumpti* colony were identical to the sequence obtained from the cultured isolate. Genome analyses revealed a typical *Borrelia* genome of 1.5 Mbp with a linear chromosome, 11 circular (range 28-169 kbp) and five linear plasmids (range 8-33 kbp). In total, the genome holds 1,500 intact genes with *vlp* loci spread across several plasmids with a linear plasmid of 25 kbp containing the majority of *vlp* genes. A *vsp* locus was identified on a linear plasmid of 33 kbp. Except for the circular megaplasmid, plasmid classification proved challenging using *PFam32* or *PFam57/62* signature genes.

Sequence comparison provides evidence that the isolate Krampitz differs from all *Borrelia* spp. for which sequence data exist and strongly supports the notion that we recovered *B. tillae*. Interestingly and in contrast to other relapsing fever species, the megaplasmid is circular. Detailed bioinformatic analyses are ongoing to characterize *B. tillae* in more detail.

OP 003

Borrelia tillae, a relapsing fever species from South Africa with human pathogenic potential

F. Schmidt[1], *S. Hepner*[2], *D. Velasquez*[2], *K. Kuleshov*[3], *G. Margos*[2], *V. Fingerle*[2], *P. Kraiczy*[1]
[1]Goethe-University Frankfurt, Institute for Medical Microbiology and Infection Control, Frankfurt, Germany
[2]German National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany
[3]Central Research Institute of Epidemiology, Moscow, Russian Federation

Borrelia tillae was first described in 1961 as a novel relapsing fever species isolated from *Ornithodoros zumpti*. The data collected in that study revealed significant differences between *B. tillae* and *B. duttonii*. These *Borrelia* species are transmitted by different vectors. Rats appear to be the main reservoir hosts of *B. tillae* and a neurotropism has been proposed in infection studies with rats and mice but no data are available regarding the pathogenicity of *B. tillae* to humans. In the present study, we sought to collect experimental evidence for factors determining *B. tillae* competence to survive in human serum and overcome complement-mediated killing. Furthermore, using whole genome data we identified and characterized a host-interacting protein of *B. tillae* partially involved in adhesion and serum resistance.

Our experimental data confirmed that *B. tillae* survived in 50 % human serum. Correspondingly, no activated complement components were detected on the spirochetal surface. Far-Western blot analyses identified distinct fibronectin-, plasminogen-, and factor H-binding proteins and further bioinformatics disclosed at least two genes encoding for host-interacting proteins in *B. tillae* on the megaplasmid. Of these, a potential fibronectin-binding protein, FbpC, sharing up to 26 % amino acid sequence identity to orthologous proteins of *B. duttonii* and *B. recurrentis*, was recombinantly produced and then functionally characterized. Our data demonstrate binding of FbpC from *B. tillae* to fibronectin and plasminogen but not to complement C1r. These findings are in contrast to the binding properties of FbpC orthologs of *B. duttonii* and *B. recurrentis* which are known to strongly interact with C1r. Consequently, FbpC exhibits an attenuated inhibitory capacity on the classical pathway due to lack of C1r binding. In conclusion, here we present first data on the capacity of *B. tillae* to evade innate immunity as an initial step to survive in the human host.

OP 004

A core genome MLST scheme for *Borrelia burgdorferi* sensu lato improves insights into the evolutionary history of the species complex

S. Hepner[1,2], *K. A. Jolley*[3], *S. Castillo-Ramirez*[4], *E. Mourkas*[5], *A. Dangel*[2], *A. Wieser*[6,7,8,9]
J. Hübner[10], *A. Sing*[1,2], *V. Fingerle*[1,2], *G. Margos*[1,2]
[1]German National Reference Center for Borrelia, Oberschleissheim, Germany
[2]Bavarian Health and Food Safety Authority, Public Health Microbiology, Oberschleissheim, Germany
[3]University of Oxford, Department of Biology, Oxford, United Kingdom
[4]Universidad Nacional Autónoma de México, Centro de Ciencias Genómicas, Programa de Genómica Evolutiva, Cuernavaca, Mexico
[5]Uppsala University, Department of Medical Sciences, Zoonosis Science Centre, Uppsala, Sweden
[6]Max von Pettenkofer Institute, Medical Microbiology and Hospital Epidemiology, Munich, Germany
[7]LMU University Hospital, Division of Infectious Diseases and Tropical Medicine, Munich, Germany
[8]German Center for Infection Research (DZIF), Partner site Munich, Munich, Germany
[9]Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Immunology, Infectious Disease and Pandemic Research IIP, Munich, Germany
[10]Dr. von Hauner Children's Hospital, Munich, Germany

The causative agents of Lyme borreliosis are bacteria of the *B. burgdorferi* sensu lato (s.l.) complex. Molecular typing is key for bacterial characterization and a multilocus sequence typing (MLST) scheme based on eight chromosomal housekeeping genes is the gold standard for *Borrelia* (https://pubmlst.org/organisms/borrelia-spp). However, availability of genome sequences enables the scale up to a genomic level. This requires extended schemes such as core genome MLST (cgMLST). To build on the success of MLST and to use the power of whole genome data, we have developed a cgMLST scheme for *B. burgdorferi* s.l.

For the cgMLST scheme development, 174 high quality *B. burgdorferi* s.l. genomes (belonging to 17 species) from the *Borrelia* PubMLST database and 815 chromosomal coding sequences of *B. burgdorferi* s.s. B31 from GenBank were used. The genomes were analyzed for loci that are present and designated in ≥95% of the genomes (resulting in 639 cgMLST loci). Phylogenetic and GrapeTree analyses based on MLST vs cgMLST were conducted for the scheme validation on 294 samples.

Phylogenetic analyses based on MLST and cgMLST showed clustering according to species but higher statistical support of phylogenetic nodes in cgMLST. Inner nodes of the MLST phylogeny were supported by bootstrap values ranging from 67–100. In the cgMLST phylogeny all internal nodes had bootstrap values of 100. Furthermore, cgMLST improved the lineage resolution within species and even for closely related isolates of the genetically homogeneous European *B. bavariensis* population.

The developed 639-loci cgMLST scheme for *B. burgdorferi* s.l. enables unambiguous genotyping and reliable phylogenetic analyses in high resolution even for closely related samples. This new scheme can be applied to chromosomal sequence data and is an addition to the 8-loci MLST scheme that is suited for environmental samples without prior culturing. Both schemes are publicly available through the *Borrelia* PubMLST website.

OP 005

Characterisation of *Borrelia burgdorferi* sequence diversity using modified multilocus sequence typing

O. N. Choi[1], *A. Woods*[2], *A. G. Marini*[3], *M. McAuliffe*[2], *A. M. Bryant*[1], *J. S. Nugent*[1], *G. M. Dill*[1]
T. F. Rounsville Jr.[1]
[1]University of Maine, Cooperative Extension, Orono, MN, United States
[2]University of Maine, College of Earth, Life, & Health Sciences, Orono, MN, United States
[3]University of Maine, School of Biology & Ecology, Orono, MN, United States

Borrelia burgdorferi sensu lato, hereafter referred to as *B. burgdorferi*, is a species complex of spirochete bacterium known to cause Lyme disease. As Lyme disease cases continue to increase in the United States, it is important to improve our understanding of the evolution and spread of this pathogen. One such method is a standardized multilocus sequence typing scheme centered on eight housekeeping genes found in *B. burgdorferi*. Here, we used a modified *B. burgdorferi* MLST framework to examine the diversity and distribution of sequence types and alleles in the state of Maine where Lyme disease is rising in incidence and also expanding geographically. DNA extracts from ticks submitted to the state tick testing program were sequenced using a new cost-effective Oxford Nanopore workflow and analyzed with a custom Python bioinformatics pipeline to identify alleles for each locus and determine sequence type. Preliminary analysis of 146 samples has identified 49 different *B. burgdorferi* sequence types amongst ticks in Maine. An additional 24 samples have potentially new sequence types not previously submitted to the pubMLST database. A BURST analysis through the pubMLST database identified eight unique clusters, the largest of which is centralized on sequence type 3 which has been previously found in the US. Hundreds more samples are currently being processed using this easily scalable method. The results of this study will improve our understanding of *B. burgdorferi*'s evolution and aid in the development of strategies to slow the expansion of Lyme disease within the state of Maine.

OP 006

Tickborne pathogen prevalence of small mammals in Pennsylvania

S. Schwartz[1], *M. Rowley*[1], *N. Chinnici*[1]
[1]East Stroudsburg University, Tick Research Lab of Pennsylvania, East Stroudsburg, PA
United States

Human pathogens vectored by ticks are a growing public health crisis across the United States. Small mammals, such as *Peromyscus* species, are among the most important reservoir hosts for tick-borne pathogens. Between 2022 and 2023, a total of 2,364 small mammals were captured across 36 sites in Northeast and Northwest Pennsylvania to assess pathogen burden in two tissue types: blood and tissue. Molecular detection of *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Babesia microti*, *Anaplasma phagocytophilum* (with variant discrimination between human active variant and variant 1), and Powassan virus (with lineage distinction between Lineage I and II) was screened using multiplex qPCR assays. The overall pathogen prevalence was higher in the Northeast compared to the Northwest. Significant co-occurrence of these pathogens, ranging from single to triple occurrences, suggest that certain pathogens are more likely to occur together rather than independently. Monitoring reservoir populations is essential to track regional variations as emerging pathogens colonize both the host and vector populations.

OP 007

Analysis of ticks (Order: Ixodida) and associated microorganisms collected on the North Sea Island of Heligoland

R. E. Rollins[1], *J. Dierschke*[1], *A. Obiegala*[2], *H. von Buttlar*[3], *L. Chitimia-Dobler*[3,4], *M. Liedvogel*[1]
[1]Institute of Avian Research “Vogelwarte Helgoland”, Wilhelmshaven, Germany
[2]University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany
[3]Bundeswehr Institute of Microbiology, Munich, Germany
[4]Fraunhofer Institute of Immunology, Infection and Pandemic Research, Penzberg, Germany

Heligoland is an island located in the North Sea, where vegetation was almost destroyed as a result of heavy bombardment during and after the Second World War. However, over the past 70 years the vegetation has developed from scrub towards bushy or even forested environments. This change has most likely altered habitat suitability for various organisms, including many species of hard ticks. Ticks can act as major vectors for various pathogens of humans and animals, thus, understanding if a tick population and associated microorganism exist on the island is of great importance in relation to public and animal health. To assess if a tick population exists on Heligoland, we flagged ticks at four different locations during June 2023-2024. In 2024, ticks were additionally sampled from house pets (n=3) living on the island and during the annual ringing of common murre (*Uria aalge*) fledglings. In total, 267 ticks were collected over the two years which were morphologically identified, and confirmed molecularly if needed, to four species: *Ixodes ricinus* (n=132), *Haemaphysalis punctata* (n=47), *Ixodes uriae* (n=3), and *Alectorobius maritimus* (n=85) which represents the first report in Germany. Questing tick samples tested positive for *Borrelia burgdorferi* sensu lato, *Rickettsia* spp., *Anaplasma phagocytophilum*, and *Babesia* spp. were found in one or both years. Subsequent sequencing showed the presence of *Rickettsia* spp. (*R. helvetica*, *R. aeschlimannii*), multiple *Borrelia* species (*B. garinii*, *B. valaisiana*, *B. bavariensis*, *B. afzelii*), and *Babesia* spp. (*B. venatorum, B. capreoli*). Our research highlights a diverse tick and associated microorganism population on the island which could pose public and animal health risks which will be monitored further.

OP 008

Impact of extreme weather events on tick-host dynamics and disease risk in mountainous regions – the study case of Vaia windstorm in the north-eastern Italian Alps

V. Tagliapietra[1,2], *G. Ferrari*[1,2], *F. Rosso*[1], *D. Arnoldi*[1], *F. Dagostin*[1], *G. Marini*[1], *A. Rizzoli*[1,2]
[1]Fondazione Edmund Mach, San Michele all'Adige, Italy
[2]National Biodiversity Future Center, Palermo, Italy

In 2018, the windstorm "Vaia" hit the North-Eastern Italian Alps causing the loss of 42 million trees across over 42,500 hectares by creating fragmented habitats that alter local animal communities and raise disease risk concerns. The area is part of the BEPREP project focusing on biodiversity recovery to reduce disease spread. This study evaluates the responses of hosts to a catastrophic event in terms of community composition and relative abundance and the consequences on zoonotic risk using small mammals, ticks and tick-borne pathogens as a model system.

The study site is located in the Paneveggio-Pale di San Martino Natural Park (Autonomous Province of Trento, Northern Italy). Twelve sites are identified in three different habitat types (post-Vaia windthrow, forest and meadow), both at 1,100 and 1,700 m a.s.l. In each site, we performed Capture-Mark-Recapture live-trapping of small mammals and tick-dragging along transects during 2023/2024. Bacteria and protozoa were detected in ticks using PCR-based methods combined with sequencing.

In total 212 *Ixodes* sp. ticks were collected through transects (97 in 2023 and 115 in 2024), with a higher tick density in Vaia sites compared to forest ones. Tick-borne pathogens screening of ticks collected in 2023 (for 2024 analyses are ongoing) reported *Anaplasma phagocytophilum* (3.33%), *Babesia venatorum* (5%), *Rickettsia monacensis* (3.33%) and *Borrelia afzelii* (20%) in Vaia sites, while in the forest only *B. afzelii* (16.22%) and *B. burgdorferi* s.s. (5.41%) were detected. We captured 261 small mammal individuals belonging to *Apodemus flavicollis*, *A. sylvaticus*, *Clethrionomys glareolus*, *Microtus* spp. and *Sorex* spp. Small mammal diversity was higher in Vaia sites.

Vaia sites are undergoing natural reforestation, with heterogeneous habitats that promote the interface between vectors and hosts, raising disease risk. This study emphasizes understanding habitat changes' ecological effects on animal communities and disease transmission.

OP 009

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

J. Kamiš[1,2], E. Richtrová[3], K. Lengálová[1,2], M. Zemanová[1], T. Arnoldová[4], J. Navrátil[3], P. Kukuliač[5]
M. Palus[1,6], H. Sprong[7], K. Kybicová[3], P. Švec[5], M. Kulma[4], V. Hönig[1,6,3]
[1]Biology Centre CAS, Institute of Parasitology, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
[3]National Institute of Public Health, National Reference Laboratory for Lyme Borreliosis, Prague, Czech Republic
[4]National Institute of Public Health, National Reference Laboratory of Pest Control, Prague, Czech Republic
[5]VSB – Technical University of Ostrava, Department of Geoinformatics, Ostrava, Czech Republic
[6]Veterinary Research Institute, Emerging viral infections group, Brno, Czech Republic
[7]National Institute of Public Health and Environment, Bilthoven, Netherlands

Tick-borne diseases are the most widespread vector-borne diseases in Central Europe. Although mainly found in forested areas, ticks also colonize urban green areas. While previous studies have focused on a few study sites and pathogens, our study investigates tick abundance and prevalence of a panel of pathogens in urban areas across the Czech Republic.

Exophilic ticks are sampled in urban green areas of 13 cities. Three sites are sampled monthly, the remaining ten once a year. Ticks are screened for tick-borne bacterial and viral pathogens using multiplex (RT-)qPCRs. Positive samples are characterized by sequencing, additional data are used to identify factors influencing tick abundance and pathogen prevalence.

Ticks (almost exclusively *Ixodes ricinus*) were found at all sampled sites with a high relative abundance of 12.7 ticks/100 m^2 (range 3.5-71.9) in 2023 and 19.3 ticks/100 m^2 (4.0-77.5) in 2024. In the samples from 2023 (2259 ticks, at least 100 per site tested), *B. burgdorferi* s.l. was detected at all 10 study sites with a high prevalence (25.9% overall, range 15.2-30.7%). *A. phagocytophilum* (7.8% overall prevalence) and *Rickettsia* sp. (6.0%) were also found at all sites, but with varying prevalence (0.9-19.0% and 3.4-12.7%, respectively). *B. miyamotoi* and *N. mikurensis* were only present in some of the areas with an overall prevalence of 2.4 and 2.3%, respectively. *B. afzelii*, *B. garinii* and *B. valaisiana* are the predominant genospecies of *B. burgdorferi* s.l. Ecotypes 1 and 2 predominate in the *A. phagocytophilum*-positive samples, while *Rickettsia* spp. are mainly represented by *R. helvetica*. No viral pathogens (tick-borne encephalitis, Kemerovo, Alongshan virus) have been found to date.

To summarize, ticks occur in the urban green spaces of Czech cities in an abundance comparable to natural habitats. The prevalence of *B. burgdorferi* s.l. is even higher than the average in natural forest areas. Thus, urban green spaces pose a significant risk to human health.

OP 010

New patterns in seasonal activity of two epidemiologically important ticks in Slovakia, *Ixodes ricinus* and *Dermacentor reticulatus*, and their infection with tick-borne agents

V. Rusňáková Tarageľová[1], D. Selyemová[1], S. Purgatová[1], B. Mangová[1], M. Chvostáč[1]
Y. M. Didyk[1,2], M. Kazimírová[1], O. Zhovnerchuk[1,2], E. Špitalská[3], M. Derdáková[1]
[1]Slovak Academy of Sciences, Institute of Zoology, Department of Medical Zoology, Bratislava, Slovakia
[2]Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine
[3]Slovak Academy of Sciences, Institute of Virology of the Biomedical Centre, Bratislava, Slovakia

Distribution areas of some tick species have changed and many tick-borne diseases have emerged (or re-emerged) within the past two or three decades due to global changes. This study deals with spatial distribution and seasonal activity of *Ixodes ricinus* (IR) and *Dermacentor reticulatus* (DR) in Bratislava (south-western Slovakia). Since July 2023, questing activity of ticks has been monitored at 11 sites by monthly flagging the vegetation and in “tick gardens” (TG). Changes in the seasonal activity of ticks compared to the past are obvious. In 2023, the highest number of IR nymphs/adults was flagged in June. In 2024, peak nymph/adult activity was observed from April to June. In August, the questing activity of nymphs and adults significantly decreased. In September and October, the number of flagged nymphs and adult ticks increased again. In 2023, IR larvae predominated in July and November 2023, and in 2024 in January, July and August. Larvae were recorded in all collections except October 2023. IR, mainly larvae, were also active during the warm December 2023 and January 2024. In February and March 2024, the number of nymphs/adults increased. Questing activity of DR in TG was observed from November 2023. The peak activity was recorded in February 2024. From March to the end of June, DR activity gradually decreased to zero. Further activity was observed in September. DR in TG were active even at sub-zero temperatures and during the snow cover in December and January. In 2023, the prevalence of IR infected with *Borrelia burgdorferi* sensu lato varied from 0% to 27.6%. *Borrelia garinii*, *B. valaisiana*, *B. afzelii*, *B. lusitaniae* and *B. spielmanii* were identified. Infections with *Babesia* spp., *Rickettsia* spp. and *Anaplasma phagocytophilum* were also confirmed. Studies on changes in questing activity of ticks and on the prevalence of tick-borne pathogens show the current risk of infection with tick-borne diseases.

Acknowledgement. The research was funded by grant APVV-22-0372.

OP 011

Unravelling urban tick-borne pathogen infection risk – are rodents key reservoirs in urban settings?

J. Rapp[1], *O. Bozbey*[1], *A. Springer*[1], *C. Strube*[1]
[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hanover, Germany

Ixodes ricinus is a key vector for *Borrelia* spp., *Rickettsia* spp., and *Anaplasma phagocytophilum* in Europe. Previous studies in Hanover"s recreational areas have shown significant variations in the prevalence of these tick-borne pathogens and tick abundance, although the causes of this remain unclear. To investigate possible urban reservoir hosts, 1,754 rodents were captured during 2020/2021 in Hanover at the ten sites long-term monitored for tick infection rates.

Analysis of 1,645 rodent spleen samples by probe-based qPCR detected *A. phagocytophilum* in 1.6% of samples, with local variations from 0.5% to 8.1%. *GroEL* sequencing classified all positive samples as the non-zoonotic ecotype 3, which is associated with the rodent-adapted tick *Ixodes trianguliceps*. *Rickettsia* spp. were found in only 0.4% of spleens (0.5 to 2.77% locally), according to their suggested role as tick endosymbionts. In addition, 1,104 ear tissue samples were screened for *A. phagocytophilum* and *Rickettsia* spp. so far. *Anaplasma phagocytophilum*, was present in 0.9% of ear samples, with local prevalences ranging from 0.5 to 8.2%, whereas *Rickettsia* spp. were identified in 8.7% of samples, with local rates between 4.0% and 16.6%.

Moreover, ear tissue from 712 rodents has already been analyzed for *Borrelia* spp., with an overall prevalence of 18.4%, and site-specific rates ranging from 3.8% to 26.0%. To confirm *Borrelia* systemic infection, bladder tissue of the rodents will be examined. *Borrelia* species differentiation will follow via Reverse Line Blot hybridisation. As no correlation between local rodent and tick infection rates is evident so far, the preliminary data imply that rodents may not play a key role as reservoirs in urban settings.

OP 012

Acarological risk in urban and peri-urban ecosystems in a French region highly endemic for ticks and tick-borne diseases.

J. Stynen[1], *C. Barthel*[1], *N. Boulanger*[1,2]
[1]Université de Strasbourg, UR 3073: PHAVI: Pathogen-Host-Arthropod Vector Interactions: group Borrelia, Strasbourg, France
[2]Hôpitaux Universitaires, French National Center for Borrelia, Strasbourg, France

Ticks expand in a variety of ecosystems, particularly in urban and peri-urban areas. Identifying the ecosystems and periods at risk is essential to the implementation of preventive measures for forestry professionals and the general public.

We carried out a north-south transect in the city of Strasbourg, which lies on the Rhine alluvial plain in eastern France. We collected ticks using the dragging technique during their peak of activity from March to June in 2024. Eight sites were collected: 2 alluvial forests, 2 peri-urban forests, 3 parks and a botanical garden. Ticks, *Ixodes ricinus* and *Dermacentor reticulatus* were collected. *Dermacentor* were only collected in alluvial forests and only the species *D. reticulatus* was present. The highest tick density (DOT) was recorded in March-April, with a DOT of 6.3 ticks/100 m^2. *Ixodes ricinus* was collected in all ecosystems but at different densities. The highest DOT was 51.7 ticks/100 m^2 in May, much higher in peri-urban areas where their hosts are abundant, and more rarely in city-center parks where vegetation is more controlled.

In terms of pathogens, *Rickettsia* spp. were detected in *D. reticulatus* ticks with an infection rate of 52% [37.5-72.8]. Finally, only *Rickettsia raoultii* was detected with 29.8% [16.7-50] of ticks infected. In the case of *I. ricinus*, infection rates varied widely. Rarely found in some parks, the overall prevalence was 19.8% [12.2; 30.5]. Various *Borrelia* species were detected: mainly *B. afzelii* (51.5%), followed by *B. lusitaniae* (18.9%), *B. garinii* (12.9%), *B. valaisiana* (12.9%), *B. burgdorferi* sensu stricto (5.3%) and *B. burgdorferi* sensu lato (3.1%). Interestingly, *B. lusitaniae* was only detected in one ecosystem, an island on the Rhine, which is a protected natural area.

Following these studies, health professionals (ARS Gand Est) and the city of Strasbourg were informed. An awareness-raising campaign is currently underway for schools and the general public.

OP 013

Kinin neuropeptide play a broad diversity of biological functions in *Ixodes ricinus* physiology

L. Abuin-Denis[1], *S. Rakotobe*[2,3], *C. Ning*[2,3], *L. Šofranková* [2,3,4], *M. Vancová* [2,3,4,5], *J. Perner*[2,3,4,5]
O. Hajdusek[2,3,4,5], *L. Šimo*[2,3]
[1]ANSES – INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Animal Health, Maisons-Alfort, France
[2]ANSES – INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Maisons-Alfort, France
[3]ANSES – INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Animal health, Maisons-Alfort, France
[4]Pavol Jozef Šafárik University in Košice, Košíce, Slovakia
[5]Czech Academy of Sciences, Ceske Budejovice, Czech Republic

Neuropeptides and their receptors are key regulators of arthropod physiology, orchestrating vital biological processes such as reproduction, development, molting, behavior, and feeding. Among them, invertebrate kinin neuropeptides are particularly notable for their myotropic activities and diverse physiological roles, including hindgut contractions, diuresis, feeding, ecdysis, and the release of digestive enzymes. While kinin and its receptor have been identified in various tick species, their physiological functions remain poorly understood. In this study, we molecularly identified the transcript sequence encoding *Ixodes ricinus* kinin prepropeptide and using in situ hybridisation visualized the kinin mRNA in specific synganglion neurons and axonal projections branching within the synganglion lobes. Immunostaining with an anti-kinin antibody confirmed the staining of kinin mRNA in neuronal bodies but also uncovered dense axonal arborization on the dorso-lateral surface of the synganglion. Further analysis using immunogold transmission electron microscopy revealed that these axons terminate at the dorsal perineurium, adjacent to the acellular neural lamella. Additionally, the anti-kinin antibody identified distinct endocrine cells within the midgut and delineated axonal processes that exclusively extend to type II salivary gland acini in female *I. ricinus*. Through phylogenetic analysis, we identified a putative *I. ricinus* kinin receptor and tested its activity in a heterologous expression system using an aequorin reporter assay. Tissue-specific expression of the kinin receptor was investigated using qRT-PCR, while immunohistochemistry confirmed its presence on synganglion neurons, type II salivary gland acini, and midgut muscle cells. These findings provide valuable insights into the diverse roles of kinin in tick biology, enhancing our understanding of this neuropeptide family in arthropods and its potential impact on tick physiology.

OP 014

African swine fever virus and vector competence in soft ticks: what do we need to do to unravel the unknown

T. Lao-Gonzalez[1], *A. Rodriguez-Mallon*[1]
[1]Center for Genetic Engineering and Biotechnology, Agricultural Research Department, Havana, Cuba

African swine fever virus (ASFV), the only member of the family Asfarviridae, is the etiological agent of a devastating hemorrhagic disease of domestic pigs and wild boar, causing major economic losses and threatening food security. As the only known DNA arbovirus, ASFV is also transmitted by soft tick species of the genus *Ornithodoros*. In Eastern and Southern Africa, soft ticks of the *O. moubata* complex are the main vectors and reservoirs for ASFV in an ancient sylvatic cycle involving warthogs. However, in the Mediterranean Basin, the main biological vector of ASFV is *O. erraticus* sensu stricto. There is strong evidence that recognizes soft ticks as an important cause of re-occurrence of ASF outbreaks in European countries. On this regard, the recent spread of ASFV in Eurasian regions and the presence of ticks with the potential to be natural vectors of the disease is a major concern. Taking into account these antecedents, we performed a critical analysis about several studies that have evaluated the ability of different *Ornithodoros* species to transmit diverse ASFV strains. The findings highlight the diversity of factors underlying the vector competence of these ticks, such as the tick species/population, virus strain and viral dissemination inside the organs involved in transmission. However, there are important gaps in the understanding of the molecular mechanisms related to vector competence across different *Ornithodoros* species for ASFV strains currently circulating in Eurasia. Finally, we propose to carry out investigations using “omics” tools and microbiome studies to unravel the molecular basis of soft tick vector competence for ASFV, find molecular markers and assess how the microbiome determines the ability to transmit tick-borne pathogens.

OP 015

Tick-borne viral pathogens in ticks and human patients from the Czech Republic

J. Kamiš[1,2], *M. Kříha*[1,2,3], *I. Říhová*[1,2], *M. Dvořáková*[1], *L. Tardy*[1], *J. Elsterová*[1], *A. Chrdle*[3,4,5]
D. Teislerová[3], *D. Růžek*[1,6,7], *M. Palus*[1,7], *K. Kybicová*[8], *M. Kulma*[9], *A. Nigsch*[10], *V. Hönig*[1,7,8]
[1]Biology Centre CAS, Laboratory of Arbovirology, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
[3]Hospital České Budějovice, Ceske Budejovice, Czech Republic
[4]Royal Liverpool University Hospital, Liverpool, United Kingdom
[5]University of South Bohemia, Faculty of Health and Social Sciences, Ceske Budejovice, Czech Republic
[6]Masaryk University, Faculty of Science, Brno, Czech Republic
[7]Veterinary Research Institute, Emerging Viral Infections Group, Brno, Czech Republic
[8]National Institute of Public Health, National Reference Laboratory for Lyme Borreliosis, Prague, Czech Republic
[9]National Institute of Public Health, National Reference Laboratory of Pest Control, Prague, Czech Republic
[10]Austrian Agency for Health and Food Safety, Mödling, Austria

Ixodes ricinus ticks are known to transmit several viral pathogens that can infect humans. The tick-borne encephalitis virus (TBEV; *Flaviviridae*: *Orthoflavivirus*) is the causative agent of tick-borne encephalitis (TBE), affecting the central nervous system. Annually, approx. 693 cases of TBE are reported in the Czech Republic. In various regions of the country, the Tribeč/Kemerovo virus (TRBV; *Sedoreoviridae*: *Orbivirus*) has been serologically confirmed in patients with a diverse range of clinical symptoms.

This study aims to detect viral RNA of TBEV, TRBV in ticks sampled from 11 sites across the Czech Republic, as well as in patient sera from the Hospital České Budějovice. Moreover, the detection is focused on the Alongshan virus (ALSV), a recently identified emerging pathogenic virus found in ticks, mosquitoes, and mammals in Asia and Europe.

During the first year of the project, 12,553 ticks (pooled samples of 10 nymphs or 5 adults) were analyzed using multiplex RT-qPCR. Positive samples were confirmed via Sanger and/or whole-genome sequencing. The acquired sequences were used for phylogenetic analyses. TBEV was found at 4 sampling sites, with a minimum infection rate ranging 0.09-0.39%. TRBV was detected at 6 sampling sites (0.09-0.28%) and ALSV at 8 sampling sites (0.09-8.56%). Out of 265 serologically confirmed human TBE cases, TBEV RNA was detected in 14 patients (5.5%), mostly in sera taken during the initial phase of TBE, and 5 patients tested positive for TBEV RNA in a group of serologically negative patients, who would otherwise have remained undiagnosed.

The results confirm the focal distribution of TBEV and TRBV and mark the first detection of ALSV in the Czech Republic. Phylogenetic analyses of TBEV strains revealed remarkable genetic stability. For patient sera, our findings underscore the transient and low-level nature of TBEV viremia, with a negative correlation observed between the success of viral RNA detection and antibody titers.

OP 016

Female mice infected with *Borrelia burgdorferi* transmit maternal antibodies to their offspring that provide strain-specific protection against tick bite

A. Foley-Eby[1]*, H. Adam*[1]*, J. Hill*[1]*, M. Voordouw*[1]
[1]Western College of Veterinary Medicine, Veterinary Microbiology, Saskatoon, Canada

In North America, the multi-strain, tick-borne spirochete *Borrelia burgdorferi* sensu stricto (*Bbss*) is the main cause of Lyme disease. In rodent hosts, *B. burgdorferi* establishes a chronic infection that induces a strain-specific antibody response. While this antibody response fails to clear the pathogen from the infected host, transfer of antibodies can protect naïve hosts against infectious challenge with *Bbss*. The purpose of our study was to investigate whether female *Mus musculus* mice infected with *Bbss* transfer maternal antibodies to their offspring, whether these antibodies provide strain-specific protection, and the duration of protection.

Female mice were assigned to four different maternal infection treatments: (1) infected with strain A, (2) infected with strain I, (3) co-infected with both strains, and (4) uninfected. The two *Bbss* strains, A and I, were named after the identity of their type of outer surface protein C (OspC). Female mice were mated with uninfected males and allowed to produce offspring. Infected mothers transferred maternal anti-*Bbss* IgG antibodies to their offspring. Offspring from mothers infected with one strain had maternal antibodies that reacted strongly with the homologous but not the heterologous recombinant OspC type. Offspring were challenged with both strains via nymphal tick bite at either 6 weeks or 11 weeks of age and the strain-specific infection status of the offspring tissues was determined at necropsy. Maternal antibodies provided offspring with stronger protection against the homologous than the heterologous strain. Protective efficacy of maternal antibodies waned over time and was higher at 6 weeks than 11 weeks of age. Breakthrough infections in the face of maternal antibodies were more common in offspring from co-infected mothers compared to mothers infected with single strains. Our study suggests that maternal antibodies in offspring could influence the fitness and frequency of *Bbss* strains in nature.

OP 017

Artificial feeding of ticks – a systematic review

B. E. Mustafa[1], *A. Ghafar*[1], *G. Abbas*[1], *S. Abullah*[2], *I. Beveridge*[1], *A. Cabezas Cruz*[3], *U. W. Massaro*[4] *A. M. Nijhof*[5], *A. Jabbar*[1]
[1]The University of Melbourne, Melbourne Veterinary School, Melbourne, Australia
[2]University of Queensland, School of Veterinary Science, Faculty of Science,, Gatton, Australia
[3]ANSES – INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Maisons-Alfort, France
[4]United States Department of Agriculture, Washington State University, Animal Disease Research Unit, Agricultural Research Service,, Pullman, WA, United States
[5]Freie Universität Berlin, Institute of Parasitology and Tropical Veterinary Medicine, Berlin, Germany

Ticks act as vectors of various infectious diseases for humans and animals. To facilitate the research on ticks and tick-borne diseases (TTBDs), various animals such as sheep, rabbit, mice etc. have been used. However, in the recent past years, artificial tick feeding system (ATFS) has gained attention as an alternative. The technique also effectively addresses the 3Rs as it can effectively replace, reduce, and refine the use of animals in TTBDs research. Herein, we have systematically reviewed the current knowledge on ATFS for soft and hard ticks. A critical appraisal of 195 articles (published between 1912 and 2024) revealed that ATFS consists of capillary feeding, membrane feeding and semi-automated membrane feeding systems. Majority of studies have focussed on membrane feeding system and few studies on the other systems. Till now, ATFS has been successfully used to study the tick biology, tick-pathogen interaction (vector competence), drug discovery and developing anti-tick vaccines under controlled environmental conditions. However, the system is not without challenges and the requirement of prolonged blood feeding for hard ticks leads to enhanced entomopathogenic fungal and/or bacterial contamination of the membrane, causing tick death. Furthermore, constant optimisation and standardisation is needed for different tick species and life stages (given their varying mouthpart lengths and physiological needs during feeding). Therefore, coordinated, and focussed efforts are needed to improve the technique to maximise its utility for studying and devising appropriate control strategies against TTBDs.

OP 018

Establishing of an artificial tick feeding system as contribution to the 3Rs approach – promising results obtained with feeding medically relevant tick species

K. Elati[1]*, C. Dippel*[1]*, K. Büchel*[1]*, H. Dautel*[1]
[1]IS Insect Services GmbH, Berlin, Germany

Introduction: Since its inception in the early twentieth century, research has been conducted to develop artificial feeding systems (AFS) as an alternative to the use of laboratory animals in rearing different hematophagous species. Several studies have been conducted in order to establish and optimize the artificial tick feeding system (ATFS) for various ixodid species including those of the genera *Ixodes, Rhipicephalus, Dermacentor* and *Hyalomma*. Because of their long feeding duration, ixodid ticks are particularly challenging for artificial feeding and still require further optimization. Based on previously published data, the objective is to further develop and establish an artificial feeding system for the rearing of ticks, thereby reducing the necessity for the use of experimental animals.

Materials and Methods: All tick species were fed on defibrinated cow blood using silicone membranes. The ticks were placed in an incubator set at 27°C and 75% relative humidity, without additional CO^2 present.

Results: This is the first report of in vitro feeding of the tick *Ixodes hexagonus*. Furthermore, the feeding of all stages of *Ixodes ricinus*, as well as the adult stage of *Hyalomma marginatum* was successfully completed. Engorgement rates reached 76.3% (29/38) and 60% (15/25) for adult *Ixodes hexagonus* and *Hyalomma marginatum*, respectively, with oviposition rates of 86.2% (25/29) and 73.3% (11/15), respectively. In vitro feeding of all stages of *Ixodes ricinus* resulted in maximal engorgement rates of 96.5% (305/316), 92.5% (37/40), and 68.4% (13/19) for larvae, nymphs, and adults, respectively.

These findings are encouraging and further feeding experiments with *Dermacentor reticulatus* and *Rhipicephalus sanguineus* are currently conducted.

OP 019

Tick-borne relapsing fever spirochetes in South America: past, present and future directions

S. Muñoz-Leal[1]
[1]Department of Animal Science, Faculty of Veterinary Sciences, University of Concepción, Chile

In South America, during early 1900s tick-borne relapsing fever (TBRF) in humans was initially detected in Colombia and Venezuela. Remarkably, *Borrelia venezuelensis*, the etiological agent, was responsible for a disease with only one febrile peak. Its vector (*Ornithodoros rudis*) was never found in the wild but always in association to human dwellings. In the meantime, *Borrelia anserina* transmitted by *Argas persicus* caused outbreaks of avian borreliosis in Brazil. Ninety years later, both spirochetes were isolated and genomic studies performed. Interestingly, *B. venezuelensis* is genetically closely related with North American *Borrelia turicatae*, yet with different plasmid signatures. Currently, even if novel TBRF spirochetes have been detected, isolation attempts have not been successful. Notwithstanding, at least three species, "*Candidatus* Borrelia Caatinga", "*Candidatus* Borrelia octodonta", and "*Candidatus* Borrelia mimona" have been characterized through multilocus genetic schemes in ticks from Brazil and Chile. Though many putative species are pending for genetic analyses in other countries of the region. Despite these occurrences, and although positive ticks do bite humans in South America, TBRF remains nowadays undiagnosed due to limited awareness and diagnostic challenges of the disease.

OP 020

Midichloria mitochondrii, a symbiont of *Ixodes ricinus* ticks, contributes to Lyme disease risk

C. Köhler[1], *M. Holding*[2], *M. Fonville*[1], *S. Moutailler*[3], *A. Heckmann*[3], *J. Zarka*[4], *E. Matthysen*[4]
A. I. Krawczyk[5,1], *H. Sprong*[1]
[1]National Institute for Public Health and the Environment, Centre for Infectious Disease Control, Bilthoven, Netherlands
[2]UK Health Security Agency, Porton Down, United Kingdom
[3]Agence Nationale de Sécurité Sanitaire, Maisons-Alfort, France
[4]University of Antwerp, Department of Biology, Wilrijk, Belgium
[5]Luxembourg Institute of Health, Department of Infection and Immunity, Esch-sur-Alzette, Luxembourg

The most abundant vertically transmitted symbiont of *Ixodes ricinus* ticks is *Midichloria mitochondrii*. Some evidence suggests its involvement in tick physiology, though its role in pathogen transmission remains unclear. We observed that questing nymphs harboring *M. mitochondrii* are coinfected with *Borrelia burgdorferi* s.l. significantly more frequently than by random chance, indicating a potential role for *M. mitochondrii* in Lyme spirochete transmission. However, this association may be confounded by the tendency of *M. mitochondrii* to occur more frequently in female ticks, as observed at the adult stage. At the larval or nymphal stages, *I. ricinus*, ticks do not show any sexual dimorphism, making it challenging to study the contribution of each sex to pathogen transmission. Here, we elucidated the roles of tick sex and *M. mitochondrii* presence in the sylvatic cycle of *B. burgdorferi* s.l. by studying immature life stages of *I. ricinus*. As no sexual marker exists for *I. ricinus*, we first developed a qPCR-assay discriminating between males and females to study *M. mitochondrii* infection in larvae and nymphs. Second, we investigated whether the sex of larvae and *M. mitochondrii* infection affect the uptake of *B. burgdorferi* s.l. during a bloodmeal on naturally infected wild birds. Third, whether the infection rate with this pathogen is higher in questing female nymphs than in male nymphs. Contrary to what has been reported for adult ticks, male and female larvae showed no difference in *M. mitochondrii* infection rates. *Midichloria mitochondrii* presence rather than tick sex-enhanced *B. burgdorferi* s.l. uptake in larvae feeding on infected birds. Similarly, *B. burgdorferi* s.l. infection in questing nymphs correlated positively with *M. mitochondrii* presence, independent of sex. This suggests that *M. mitochondrii* alters the propensity of *I. ricinus* ticks to acquire, maintain, and possibly transmit *B. burgdorferi* s.l., contributing to Lyme disease risk.

OP 021

Exploring *Ixodes ricinus* endo-microbiome transmission modes

P. Lesiczka[1], *T. Azagi*[2], *A. I. Krawczyk*[2], *W. Scott, Jr.*[3], *R. Dirks*[4], *L. Simo*[5], *G. Dobler*[6], *B. Nijsse*[3]
P. Schaap[3], *H. Sprong*[2], *J. Koehorst*[3]
[1]Nederlands Instituut voor Vectoren, Invasieve Planten en Plantgezondheid, Centrum Monitoring Vectoren, Wageningen, Netherlands
[2]National Institute for Public Health and the Environment, Centre for Infectious Diseases, Bilthoven, Netherlands
[3]Wageningen University, Laboratory of Systems and Synthetic Biology, Wageningen, Netherlands
[4]Future Genomics Technologies, Leiden, Netherlands
[5]ANSES – INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Maisons-Alfort, France
[6]Bundeswehr Institute of Microbiology, Munich, Germany

Ixodes ricinus ticks act as vectors for numerous pathogens that present substantial health threats. Additionally, they harbor vertically transmitted symbionts, some of which have been linked to diseases. The difficulty of isolating and cultivating these symbionts has hampered our understanding of their biological role, potential to cause disease, and their modes of transmission. To expand our understanding on the tick symbiont *Midichloria mitochondrii* and on *Rickettsia helvetica*, we utilized deep sequencing on sixteen individual adult female ticks collected from coastal dune and forested areas in the Netherlands.

By employing a combination of second and third-generation sequencing techniques, we successfully reconstructed the complete genomes of *M. mitochondrii* from eleven individuals, *R. helvetica* from eight individuals and the mitochondrial genome from all ticks. Additionally, we visualized the location of *R. helvetica* in tick organs and constructed genome-scale metabolic models (GEMs) of both symbionts to study their environmental dependencies.

Our analysis revealed a strong co-phylogeny between *M. mitochondrii* and mitochondrial genomes, suggesting frequent maternal transmission. In contrast, the absence of co-phylogeny between *R. helvetica* and the mitochondrial genomes, coupled with its presence in the *receptaculum seminis* of *I. ricinus* females, raises the possibility of paternal transmission of *R. helvetica*.

Notably, the genetic diversity of *R. helvetica* was found to be very low, except for the *rick*A gene, where the presence of up to thirteen insertions of a 33nt-long repeat led to significant variability. However, this variation could not account for the differences in infection prevalence observed across locations in the Netherlands.

OP 022

Complex interactions between two lineages and two symbionts in *Ixodes frontalis*

S. Melis[1], *L. Gammuto*[1], *M. Castelli*[1], *T. Nardi*[2], *B. Bisaglia*[1], *O. Duron*[3], *E. Olivieri*[4], *H. Sprong*[5]
O. Plantard[6], *D. Sassera*[1,7]
[1]University of Pavia, Pavia, Italy
[2]Ospedale Maggiore Policlinico, Clinical Sciences and Community Health, Milan, Italy
[3]University of Montpellier, Montpellier, France
[4]Istituto zooprofilattico della Lombardia e dell'Emilia Romagna, Pavia, Italy
[5]National Institute for Public Health and the Environment, Utrecht, Netherlands
[6]INRAE, Animal Health division, BIOEPAR lab, Nantes, France
[7]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Introduction: *Ixodes frontalis* is a neglected ornithophilic tick species widely distributed in Europe. Hornok and colleagues (2016) showed that two genetic lineages, namely haplotype A and B, are clearly recognizable in the populations of this tick species, based on differences in COI gene. Little is known about the bacterial symbionts of *I. frontalis*, despite symbionts of other ticks having been investigated and shown to have fundamental roles for tick survival and blood metabolism. We aimed to investigate the relative abundance of the two different lineages and the presence of symbionts.

Materials and Methods: *Ixodes frontalis* specimens were collected in different areas in France and Italy. Presence and load of *Midichloria*, *Spiroplasma* and *Rickettsia* were investigated. COI mitochondrial gene PCRs and sequencing were also performed to discriminate between the two lineages. Furthermore, a correlation analysis was run to compare the molecular data of the symbionts with the tick haplotypes. Finally, WGS was used to obtain complete mitochondrial genomes of the two haplotypes, as well as *Spiroplasma* and *Midichloria* representative genomes.

Results: In the investigated locations, haplotype A appeared to be more common than B (A:74%, B:26%). *Midichloria* and *Spiroplasma* symbionts were present in all adult specimens (21% *Midichloria*, 33% *Spiroplasma*, 46% both symbionts), while *Rickettsia* was not detected. No correlation between haplotype and symbiont presence was found. Genomics reveals that at the mitogenome level the two haplotypes differ by 8%. *Midichloria* retains genes for B vitamins biosynthesis, while *Spiroplasma* possesses genes potentially involved in the defense of the host. Our results paint a picture of complex interactions, where two well distinct lineages interact with two symbionts. Open questions include: Are the two haplogroups cryptic species? What is the role of the two symbionts? Are they competing or possess complementary functions?

OP 023

Innovative approaches to vector vaccination – targeting the microbiota to prevent vector-borne diseases

A. Cabezas Cruz[1]
[1]INRAE, Sante Animale, Paris, France

Vector-borne diseases remain a significant threat to public and animal health worldwide. Traditional approaches to controlling these diseases have focused on targeting pathogens directly or reducing vector populations through chemical interventions. However, emerging strategies that target the vector's microbiota offer a novel and promising avenue for disease prevention. In this talk, I will present recent advances in the development of antimicrobiota vaccines that disrupt the symbiotic relationships between vectors, such as ticks, and their microbiota. By altering the microbial environment within vectors, these vaccines can reduce pathogen transmission and vector competence, ultimately preventing disease in the host. We will explore the underlying mechanisms, discuss potential vaccine targets, and highlight the implications of this innovative approach for the future of vector control.

OP 024

Near-complete bacterial genome insertion in a tick nuclear genome – evidence from tick cell lines and ticks

J. J. Khoo[1], *A. Beliavskaia*[1], *M. Whitehead*[1], *C. Hartley*[1], *A. Al-Khafaji*[1], *G. Ward*[1], *S. Armstrong*
G. Bah[2], *M. Kazimírová*[3], *A. Darby*[1], *B. Makepeace*[1], *L. Bell-Sakyi*[1]
[1]University of Liverpool, Institute of Infection, Veterinary and Ecological Sciences, Liverpool, United Kingdom
[2]Institut de Recherche Agricole pour le Développement, Centre de Recherche Agricole de Wakwa, Ngaoundéré, Cameroon
[3]Slovak Academy of Sciences, Bratislava, Slovakia

In earlier screening for bacterial endosymbionts in tick cell lines at the Tick Cell Biobank, horizontal gene transfer (HGT) of bacterial genes into the tick genome was initially suspected when cell lines generated from the tropical bont tick *Amblyomma variegatum* yielded positive PCR amplification of genes from the pathogen *Rickettsia africae*, known to be transmitted by this tick, even though light and electron microscopic examination of the cells did not show any signs of bacterial infection. Here we describe the investigations leading to the discovery of an insertion of the nearly complete *R. africae* genome into the *A. variegatum* nuclear genome.

Tetracycline treatment of *A. variegatum* cell lines AVL/CTVM13 and AVL/CTVM17 did not deplete the *Rickettsia*-specific *gltA* gene when examined by quantitative PCR, and proteomics analysis of both lines failed to identify any *Rickettsia*-specific proteins, suggesting absence of bacteria. Next generation sequencing of AVL/CTVM17 cells provided evidence for the insertion of a nearly complete *R. africae* chromosome (~1.2Mb) into the tick genome, although a ~54kb region containing genes essential for *Rickettsia* bacterial metabolism was absent. Sequence of the pRa plasmid, which is normally found in live *R. africae*, was also not detected. We conducted further analyses of the genome sequence coverage, single nucleotide polymorphism profile, and plasmid gene copy numbers from the cell line, *A. variegatum* ticks from a *Rickettsia*-free colony, and ticks from the field, giving further evidence for the genetic differences between the *R. africae* genome insertion and *R. africae* bacteria infecting ticks.

These findings represent the first confirmation of a pathogen chromosome integrated into the genome of its vector and highlight the potential impact of bacterial genome insertion on pathogen or endosymbiont surveillance in arthropods. We also demonstrate the value of arthropod cell lines in facilitating research on HGT in arthropod genomes.

OP 025

Academic publishing: doom – Or dawn of a new era?

U. Dirnagl[1]
[1]QUEST Center for Responsible Research, Berliner Institut für Gesundheitsforschung und Abteilung für Experimentelle Neurologie, Charité Universitätsmedizin Berlin, Germany

Scientific publishing is a system in need of significant reform. Emerging approaches hold promise for transformation, but meaningful change depends on the commitment of institutions, funders, and researchers to challenge the current norms. The current model often operates as an oligopoly, leveraging researchers' work to generate substantial profits while creating a marketplace of curated articles. This has led to an overwhelming volume of under-utilized publications, driven by publish-or-perish pressures and further complicated by the rise of predatory journals enabled by AI. While Open Access is an ethical imperative, its potential has been undermined by high Article Processing Charges that prioritize profit over accessibility. Peer review, though foundational, is often inefficient, prone to bias, and lacks transparency, with cascading review processes causing delays that primarily benefit publishers. Additionally, publication bias continues to obscure null results, limiting the completeness of scientific knowledge. Moving forward, solutions such as preprints, registered reports, open peer review, FAIR data practices, and a rethinking of academic incentives are essential to restoring the integrity and efficiency of scholarly publishing.

OP 026

Pericytes and microglia in tick-borne encephalitis pathogenesis and blood-brain barrier dynamics

V. Prančlová[1,2], M. Čížková[1,2], M. Vancova[1,2], V. Hönig[1,3], M. Davídková[1], M. Dvořáková[1]
E. Kotounova[1,2], D. Růžek[1,3,4], M. Palus[1,3]
[1]Biology Centre CAS, Laboratory of Arbovirology, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
[3]Veterinary Research Institute, Department of Virology, Brno, Czech Republic
[4]Masaryk University, Department of Experimental Biology, Brno, Czech Republic

Tick-borne encephalitis virus (TBEV), a neurotropic flavivirus, causes severe CNS infections characterized by neuroinflammation and blood-brain barrier (BBB) dysfunction. While neurons are primary targets, the roles of pericytes, microglia, and TBEV nonstructural protein (NS1) in disease progression are increasingly recognized.

Pericytes, crucial components of the neurovascular unit (NVU), support BBB maintenance and regulate neuroinflammation. Infection of primary human perivascular pericytes with virulent (Hypr) and mildly virulent (Neudörfl) TBEV strains demonstrated productive replication without cytopathic effects. However, infection significantly elevated IP-10, RANTES, MCP-1, and IL-6 levels, implicating pericytes in shaping the inflammatory milieu that may indirectly compromise BBB function.

Microglia, CNS-resident immune cells, sustained long-term TBEV infections across strains, with the most virulent strain eliciting the highest cytokine induction (IP-10, IL-6, and MCP-1). Strain-specific immune responses were accompanied by ultrastructural changes, including autophagic vacuoles, suggesting microglia's dual role in viral persistence and neuroinflammation.

Furthermore, TBEV NS1 protein was shown to enhance permeability in human brain microvascular endothelial cells. Together, pericytes and microglia contribute to TBEV-induced neuroinflammation, with pericytes acting as sources of key signaling molecules and microglia amplifying immune responses. The interplay between these cell types and impact of TBEV NS1 protein highlights their plausible roles in BBB dysfunction and neuropathology, providing critical insights into TBE pathogenesis and potential therapeutic targets.

This integrative approach underscores the importance of both cellular and viral factors in modulating BBB integrity and CNS inflammation during TBEV infection.

OP 027

Chemokine-binding proteins in *Ixodes ricinus* saliva

J. Kotál[1], *W. Nazish*[2], *K. Hronková*[1], *K. Suchá*[1], *C. Curcă*[2]
[1]University of South Bohemia, Dept. of Medical Biology, Ceske Budejovice, Czech Republic
[2]University of Applied Sciences, Krems, Austria

Ixodes ticks are important disease vectors and their impact on public health is rapidly growing. The feeding success, as well as vectorial capacity of *I. ricinus* depend on the immunomodulation of the tick bite site. The host immune system represents a robust defense where cytokines and chemokines regulate immunological processes like inflammation, cell adhesion, migration, activation or release of other cytokines. To modulate the immune response, ticks secrete a repertoire of salivary molecules interfering with cytokine production or by directly inactivating cytokines. Regulation of cytokine production by various mechanisms has been reported for a multitude of tick species. However, molecules directly binding cytokines have not been studied in such depth. In fact, cytokine binding activity has only been characterized in detail for Evasins from *Rhipicephalus sanguineus*. Evasin homologues were subsequently characterized also in *Amblyomma spp., I. ricinus,* and *R. pulchellus*. However, they are present in transcriptomes of numerous other tick species.

Saliva of *I. ricinus* and its role in tick-host-pathogen interaction has been studied with great detail, compared to most other tick species. However, the specific role of cytokine binding molecules in *I. ricinus* saliva remains poorly understood. In *I. ricinus*, only few homologues of Evasins have been partially characterized. Apart from Evasin homologues that bind CC and CXC chemokines, we lack knowledge about proteins binding other cytokines. Saliva of *I. ricinus* inhibits detection of IL-2, IL-4, IL-8, and TNF by ELISA and therefore binds these cytokines. However, the specific molecules causing this effect are unknown.

Here we present homologues of Evasin-3 from *I. ricinus* saliva. We confirmed their chemokine-binding activity and tested their role in cell migration and cell adhesion assays. Our results provide better insight into tick-host interaction and can be applicable as potential pharmaceuticals or vaccine candidates.

OP 028

Changes in the genetic structure of population of the three tick-borne encephalitis virus strains representing the main virus subtypes during persistence in different tick species

A. Polienko[1], *A. Gladkikh*[2], *O. Belova*[1], *A. Litov*[1], *E. Klyuchnikova*[2], *V. Dedkov*[2], *G. Karganova*[1]
[1]Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Sciences (Institute of Poliomyelitis), Moscow, Russian Federation
[2]Saint Petersburg Pasteur Institute, Saint Petersburg, Russian Federation

The tick-borne encephalitis virus (TBEV) is a causative agent of a dangerous arboviral infection. TBEV is predominantly transmitted by *Ixodes persulcatus* and *I. ricinus* ticks, while the secondary vectors are ticks of the genus *Dermacentor*. There are three main subtypes of the virus, which differ in their pathophysiological characteristics: European (Eu), Siberian (Sib), Far-Eastern (FE). Recently, TBEV subtypes have increasingly been identified in atypical tick species. Vector switch may lead to the emergence of the virus with increased virulence for humans. The study of microevolutionary processes in different hosts can provide insights into the mechanisms of the virus adaptation to a new host.

To study the changes in the TBEV properties, we performed a persistent infection in *I. ricinus, I. persulcatus*, and *D. reticulatus* for 35-42 days with one strain for each of the main subtypes, resulting in 15-18 variants of the each subtype. Infectivity in the PEK cell culture, neuroinvasiveness in mice, and single nucleotide variations (SNV) in the structural region of the genome were assessed for every obtained variant.

A decrease in infectivity was observed for all obtained variants, while neuroinvasiveness was reduced only for the FE subtype, irrespective of the tick species to which the virus was adapted. Across all Eu variants, 28 SNV were identified (15 non-synonymous (ns)), for Sib - 21 SNV (11 ns), and for Fe - 15 SNV (7 ns). All Eu variants had 3 ns SNVs at the same site, and a correlation was found between the accumulation of mutations in the protein E and infectivity after adaptation to *I. ricinus* and *D. reticulatus*. The reduction in infectivity of the Sib and FE subtypes appears to be associated with SNVs in the non-structural regions of the genome.

In conclusion, the Eu strain exhibited the highest genetic variability in adaptation to different tick species. Viruses of varying subtypes may differ in sites responsible for infectivity in mammalian cells.

OP 029

A spatio-temporal modeling framework to assess the probability of human tick-borne encephalitis (TBE) infections across Europe

F. Dagostin[1], *D. Erazo*[2], *G. Marini*[1], *D. Da Re*[1,3], *V. Tagliapietra*[1], *M. Avdicova*[4], *T. Avšič – Županc*[5]
T. Dub[6], *N. Fiorito*[7], *N. Knap*[5], *C. M. Gossner*[8], *J. Kerlik*[4], *H. Mäkelä*[6], *M. Markowicz*[9], *R. Olyazadeh*[10], *L. Richter*[9], *W. Wint*[10], *M. G. Zuccali*[11], *M. Žygutienė*[12], *S. Dellicour*[2], *A. Rizzoli*[1]
[1]Fondazione Edmund Mach, Research and Innovation Centre, San Michele all'Adige, Italy
[2]Université Libre de Bruxelles, Spatial Epidemiology Lab, Bruxelles, Belgium
[3]University of Trento, Center for Agriculture Food Environment, Trento, Italy
[4]Regional Authority of Public Health in Banská Bystrica, Banská Bystrica, Slovakia
[5]University of Ljubljana, Institute of Microbiology and Immunology, Faculty of Medicine,, Ljubljana, Slovenia
[6]Finnish Institute for Health and Welfare, Department of Health Security, Helsinki, Finland
[7]Unità Locale Socio Sanitaria Dolomiti, Belluno, Italy
[8]European Centre for Disease Prevention and Control, Stockholm, Sweden
[9]Austrian Agency for Health and Food Safety, Vienna, Austria
[10]Environmental Research Group Oxford Ltd, Dept Biology, Oxford, United Kingdom
[11]Azienda Provinciale Servizi Sanitari, Dipartimento di Prevenzione, Trento, Italy
[12]National Public Health Center under the Ministry of Health, Vilnius, Lithuania

Background: Tick-borne encephalitis (TBE) is a viral disease of the central nervous system caused by the tick-borne encephalitis virus (TBEV). Despite the availability of a vaccine, TBE incidence is increasing with new foci of virus circulation. Within this context, modeling the occurrence of human TBE cases at the finest scale is essential to support targeted public health interventions. In response, this study presents a novel spatio-temporal modelling framework that provides annual predictions of human TBE presence in Europe.

Methods: We used a boosted regression tree model trained on TBE data provided by the European Surveillance System (TESSy, ECDC) during the period 2017-2022. To account for the natural hazard of viral circulation, we included variables related to temperature, precipitation, land cover, and tick host presence. We also used proxies for human outdoor activity in forests and population density to account for human exposure to tick bites.

Results:Our results highlight a statistically significant rising trend in the probability of human TBEV infections in north-western and south-western Europe. Such areas are characterised by the presence of key tick host species, forested areas, intense human recreational activity in forests, steep drops in late summer temperatures and high precipitation amounts during the driest months. The model showed good predictive performance, with a mean AUC of 0.85 at the regional level, and a mean AUC of 0.82 at the municipal level.

Discussion: With ongoing climate changes, the burden of human TBEV infections on European public health is likely to increase. Hence, the development of a modeling framework that estimates the probability of human TBEV infections at the finest scale represents a step forward towards comprehensive TBE risk estimation. Our model will aid in identifying areas suitable for targeted TBEV detection surveys and support public health authorities in planning surveillance and prevention efforts.

OP 030

Thermosensitivity of TBE virus strains – experimental studies

A. Lindau[1], *K. Fachet-Lehmann*[1], *G. Dobler*[1,2], *U. Mackenstedt*[1]
[1]University of Hohenheim, Department of Parasitology, Stuttgart, Germany
[2]German Armed Forces, Bundeswehr Institute of Microbiology, Munich, Germany

Climate change is altering vector-borne disease dynamics, with rising temperatures increasingly affecting pathogen transmission and virulence. Tick-borne encephalitis virus (TBEV), a significant public health concern across Europe, is primarily transmitted by *Ixodes ricinus* ticks. Understanding the factors that influence TBEV replication and persistence within its vector is crucial for accurately predicting disease risk. Based on the findings of Elväng et al. (2011), who demonstrated a thermosensitive RNA-switch within the 3”-UTR of the TBEV strain Torö-2003 that modulates virus replication in response to temperature changes, this study investigates the thermosensitivity of various TBEV strains to assess the impact of rising temperatures on viral persistence and replication in *I. ricinus*. Specifically, we examined a meadow isolate and a forest isolate, both from a known hotspot in Bavaria, and the Neudoerfl strain as a control. Infected *I. ricinus* nymphs were incubated at temperatures of 22°C, 27°C, 32°C, and 37°C over a period of up to 12 weeks. Viral load and detectability were assessed using RT-qPCR and plaque assays, while mutations within the 3”-UTR that could potentially influence thermotolerance were analyzed. Results showed that high temperatures significantly reduced viral loads and detectability of the meadow isolate, whereas the forest isolate displayed thermotolerance similar to Neudoerfl, suggesting a common mechanism. Prolonged exposure of the meadow isolate to 32°C and 37°C indicated increased genetic variability, pointing to a shift toward non-infectious particles. These findings underscore the need for further studies on climate-induced selection within TBEV strains and the implications for pathogen evolution in tick populations.

Elväng et al., Sequencing of a tick-borne encephalitis virus from Ixodes ricinus reveals a thermosensitive RNA switch significant for virus propagation in ectothermic arthropods. Vector Borne Zoonotic Dis. 2011 Jun;11(6).

PP 001

Structural and functional characterisation of the β-barrel assembly machinery complex from the Lyme disease causing agent *Borrelia burgdorferi*

K. Brangulis[1]
[1]Latvian Biomedical Research and Study Centre, Tick-Borne Pathogens Research Group, Riga, Latvia

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, poses a significant health threat in Europe and the United States. Understanding the molecular mechanisms underlying its pathogenesis is crucial for the development of effective therapeutic strategies. This study focuses on the β-barrel assembly machinery (BAM) complex, an essential component of the outer membrane in Gram-negative bacteria, including *B. burgdorferi*. The BAM complex plays a critical role in the folding, assembly, and export of outer membrane proteins. The aim of this research is to provide valuable insights into the spirochete's biology by unraveling the formation and function of the BAM complex. Recently, we solved the crystal structure of the BAM complex protein BamB, which adopts a β-propeller structure composed of seven β-sheets arranged in a circular structure, with each β-sheet consisting of four anti-parallel β-strands. Our research goal is to employ multidimensional studies to fully characterize the BAM machinery in *B. burgdorferi*, focusing on protein-protein interaction analysis and the determination of 3D structures of protein-protein complexes. This work is supported by the ANM grant Nr. 26/BMC/PG.

PP 002

Evolution of the vls antigenic variation system in Lyme *Borrelia* genomes

L. Li[1], *A. Collado*[1,2], *L. Di*[1], *E. Mongodin*[3], *R. Morgan*[4], *S. Schutzer*[5], *C. Fraser*[3], *B. Luft*[6]
S. Casjens[1], *W. Qiu*[1,2]
[1]Hunter College, Computational Biology, New York, NY, United States
[2]Weill Cornell Medical College, New York, NY, United States
[3]University of Maryland, Baltimore, MD, United States
[4]New England BioLabs, Ipswich, MA, United States
[5]Renaissance School of Medicine, Stonybrook, Long Island, NY, United States
[6]University of Utah School of Medicine, Utah, UT, United States

The genomes of *Borrelia* bacteria associated with Lyme disease possess a highly variable multi-copy gene locus known as *vls*, which enables antigenic variation within hosts. To gain insights into the adaptive evolution of the *vls* gene system, we inferred within- and between-species phylogenies of the *vls* tandem repeats in Lyme *Borrelia* genomes. Using the *vls* genes re-annotated and identified by Norris and Brangulis (2024) from 31 *Borrelia* genomes, our analysis revealed concerted evolution at the *vls* locus, where sequence similarity within a strain exceeded that between strains. Meanwhile, phylogenetic inconsistencies were observed between species, geographic groups, and pathogenic types, suggesting horizontal gene transfer and incomplete lineage sorting. Moreover, the *vls* system displayed significant gains and losses in repeat copy numbers accompanying strain and species divergence. Results from the McDonald-Kreitman test indicated that *vls* diversity is likely driven by diversifying selection acting on the variable regions of the cassettes, consistent with their roles in escaping host adaptive immunity. Ancestral reconstruction analysis unveiled multiple translocation events of the *vls* locus among plasmids. To validate these evolutionary mechanisms underlying the *vls* gene evolution, we developed a custom simulation program. The simulation results suggested that the concerted evolution pattern of *vls* is likely maintained by a combination of recombination (in the form of gene conversion) and frequent duplication and loss events. Our study reveals that a complex interplay of evolutionary mechanisms, including balancing selection, gain/loss events, recombination, and plasmid transfer, contributes to the observed diversity and variability in the *vls* system. These findings revealed the evolutionary mechanisms of antigenic variation in *Borrelia* and provide an analytical framework for exploring the adaptive evolution of similar antigen variation systems in other microbial pathogens.

PP 003

Optimising Transformation Efficiency in *Borrelia* using in vitro methylated plasmids to bypass the restriction-modification system

M. Ruivo[1]*, N. Zsuzsa Kovács*[1]*, A. M. Schötta*[1]*, T. Stelzer*[1]*, L. Hermann*[1]*, V. Mündler*[1]
A. Bergthaler[1]*, M. Reiter*[1]*, M. Wijnveld*[1]
[1]Institute for Hygiene and Applied Immunology - Medical University of Vienna, Vienna, Austria

Borrelia is transmitted to humans by the bite of an infected tick. In Europe, *B. afzelii* and *B. garinii* are the main causative agents of Lyme borreliosis, one of the most prevalent tick-borne diseases. A *Borrelia* defence mechanism is the restriction-modification system (RMS), which protects against the introduction of foreign DNA into the cells. This system comprises two proteins: endonuclease and methyltransferase. We aimed to bypass this system by in vitro methylating the plasmids before electroporation in different isolates of *Borrelia.*
For in vitro methylation, the plasmid pBSV2_OspA_GFP was co-transformed into a *dam-/dcm- Escherichia coli* strain containing RMS for *Borrelia*. This way, the plasmid is methylated by the methyltransferase expressed in this strain. The methylated plasmid was used to transform *B. burgdorferi* B31 and *B. afzelii* and *B. garinii* patient isolates. Unmethylated pBSV2_OspA_GFP was used as a control.

A substantial increase in transformation efficiency was observed when the plasmids were methylated prior to transformation both in lab and patient isolates. In *B. burgdorferi* B31, we observed that 16% of the viable spirochetes were successfully transformed when a methylated plasmid was used, compared to only 7% for unmethylated plasmids. With patient isolates, 14% and 22% of the viable *B. afzelii* and *B. garinii*, respectively, were successfully transformed with methylated plasmid in comparison to 2% and 0% with unmethylated plasmid.

The increase observed by using in vitro methylated plasmids may be due to the plasmid being recognised by the RMS of the borrelial strain as host DNA and, thus, is not degraded by the endonuclease. Our study demonstrates that bypassing the RMS defences is necessary to increase transformation efficiency. The knowledge gained will allow further host-pathogen and tick interaction studies. It could potentially lead to the development of a targeted strategy to cure Lyme borreliosis based on bacteriophages.

PP 004

Adhesion of *Borrelia* and other human pathogenic bacteria to endothelial cells is facilitated by fibronectin interaction

D. J. Vaca[1], *F. Frenzel*[1], *W. Ballhorn*[1], *S. Garcia Torres*[1], *M. S. Leisegang*[2,3], *S. Günther*[4]
D. Bender[5], *P. Kraiczy*[1], *S. Göttig*[1], *V. A. J. Kempf*[1]
[1]Goethe-University Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt a.M., Germany
[2]Goethe-University Frankfurt, Institute for Cardiovascular Physiology, Frankfurt a.M., Germany
[3]German Center of Cardiovascular Research (DZHK), Frankfurt a.M., Germany
[4]Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
[5]Federal Institute for Vaccines and Biomedicines, Langen, Germany

Human pathogenic bacteria circulating in the bloodstream need to find a way to interact with endothelial cells (ECs) lining the blood vessels to infect and colonise the host. The extracellular matrix (ECM) of ECs might represent an attractive initial target for bacterial interaction, as many bacterial adhesins have reported affinities to ECM proteins, in particular to fibronectin (Fn). Here, we analysed the general role of EC-expressed Fn for bacterial adhesion. For this, we evaluated the expression levels of ECM coding genes in different ECs, revealing that Fn is the highest expressed gene and thereby, it is highly abundant in the ECM environment of ECs. The role of Fn as a mediator in bacterial cell-host adhesion was evaluated in adhesion assays of *Acinetobacter baumannii*, *Bartonella henselae*, *Borrelia burgdorferi*, and *Staphylococcus aureus* to ECs. The assays demonstrated that bacteria colocalised with Fn fibres, as observed by confocal laser scanning microscopy. Fn removal from the ECM environment (*FN1* knockout ECs) diminished bacterial adherence to ECs in both static and dynamic adhesion assays to varying extents, as evaluated via absolute quantification using qPCR. Interactions between adhesins and Fn might represent the crucial step for the adhesion of human-pathogenic Gram-negative and Gram-positive bacteria targeting the ECs as a niche of infection.

PP 005

Early pathogenic mechanisms of Lyme disease: Volume electron microscopy insights into *Borrelia burgdorferi* barrier penetration and spread

M. Vancová[1], *M. Strnad*[1], *J. Týč*[1], *F. Kitzberger*[1], *J. Kopecká*[1], *R. Rego*[1]
[1]Biology Centre of the CAS, Institute of Parasitology, Ceske Budejovice, Czech Republic

The Lyme disease spirochete *Borrelia burgdorferi* is transmitted through the Ixodes ticks to the dermis of a mammalian host. Hematogenous and non-hematogenous routes further disperse the spirochete to distal tissues. However, the detailed mechanisms of dissemination and invasion are not well understood.

In this study, 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 2 hours after intravenous and/or subcutaneous injection of *Borrelia burgdorferi sensu stricto* in the presence or absence of *Ixodes ricinus* ticks feeding in neighboring areas. 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 comprehensive approach allows us to study 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.

PP 006

Retention of lp56 in *Borrelia burgdorferi* transposon mutants and investigating the importance of the bactofilin *fapA* (BB0267) for infection

N. Hagleitner[1], *M. Vancová*[1,2], *P. Rosa*[3], *R. Rego*[1,2]
[1]Institute of Parasitology, Biology Centre, CAS, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Department of Chemistry, Ceske Budejovice, Czech Republic
[3]Rocky Mountain Laboratories, NIAID, NIH, Laboratory of Bacteriology, Hamilton, MT, United States

Many efforts have been made to identify genes and the proteins they encode that are important in the biology and pathogenesis of Lyme disease *Borrelia*. One of these methods used was transposon mutagenesis of the *B. burgdorferi* infectious strain 5AN, lacking the two restriction-modification genes *bbe02* and *bbq67*. Although *bbe02* was inactivated in this background, the entire plasmid lp56 is absent (on which is present *bbq67*). Given studies in the past highlighting the importance of certain genes on lp56 for infection and persistence (*ErpX* and p35) and more recently in dissemination in human patients, it would be important to obtain transposon mutants in a background that retains all plasmids including lp56. To this end we carried out transformation of the *B. burgdorferi* B31-S4 strain (Dbbe02) using the transposon vector pGKT. Prior to transformation, the plasmid DNA was methylated in vitro using CpG methyltransferase. Unlike previous studies we were successful in obtaining transposon mutants, all retaining lp56 besides the other plasmids. Upon identifying the mutations, we investigated the mutant for fapA (bb0267). This mutant revealed defects in growth rate, morphology, motility and infectivity. BB0267 lacking spirochetes grew significantly slower and showed a decreased ability to move through the soft agar medium in comparison to wild type B31-A3. Furthermore, electron microscopic pictures revealed spirochetes with altered helical morphology, often forming hook or loop-like structures at one end of the protoplasmic cell cylinder. Infectivity studies revealed a reduced infectivity phenotype of BB0267 mutants. In tick infection studies, BB0267 mutants were not acquired during feeding of naive tick larvae on infected mice, suggesting an acquisition phenotype for this transposon mutant. Complementation studies are currently ongoing. This is the first successful study to obtain *Borrelia* transposon mutants that retained lp56 and may be more relevant for future studies.

PP 007

Bites of knowledge – citizen science focused on schools to increase awareness and knowledge on ticks

J. Millán[1], R. Rodríguez-Pastor[2], C. Muñoz-Hernández[3], I. Garcia-Fernández de Mera[3]
M. Sánchez-Sánchez[3], A. Estrada-Peña[4]
[1]Fundación ARAID, Zaragoza, Spain
[2]Universidad de Zaragoza, Salud Animal, Zaragoza, Spain
[3]Instituto de Investigacion en Recursos Cinegéticos, SaBio, Ciudad Real, Spain
[4]Ministerio de Salud Humana, Madrid, Spain

Knowledge about the distribution of ticks and transmitted pathogens is of most importance for evaluation of impact and mitigation measures. Citizen science is the active engagement of the public in scientific tasks. Regarding ticks, citizen collaboration can capture the epidemiological patterns behind transmitted pathogens. This study focused citizens in the region of Aragón (north-east Spain), improving their knowledge on ticks and providing specimens for further studies.
We recruited 20 rural schools (students 12-16 years old), a number of hunters, rangers, and other volunteer citizens. The research team visited the schools introducing a short video and a talk about tick collection and preservation. A kit was supplied to the teachers at each school (flyer, tubes, alcohol, forceps, etc.). Both hunters and rangers received a simplified kit. Ticks were sent to our laboratory, morphologically identified, and submitted to DNA extraction and identification of carried pathogens.

Nineteen schools provided material labelled and in agreement with minimum requirements. Overall, 921 ticks were collected: 375 (schoolchildren) 320 (hunters) 108 (rangers) 91 (general citizens) and other 27 randomly by the authors. We identified *Rhipicephalus sanguineus* s.l. (n = 303), *R. sanguineus* s.s. (64), *R. pusillus* (337), *R. bursa* (86), *Dermacentor marginatus* (27), *Ixodes hexagonus* (6), *I. ricinus* (13), *I. frontalis* (1), *Haemaphysalis punctata* (16), *H. sulcata* (3), *H. inermis*, (1), *Hyalomma marginatum* (17), and *H. lusitanicum* (4). Molecular screening pinpointed *Rickettsia massiliae*, *R. aeschlimannii*, *R. helvetica* and *R. conorii raoultii*, in different tick species, as well as 11.53% of *Borrelia* spp. in feeding *I. ricinus* ticks.

The interest of children in rural schools is high towards ticks. Together with rangers and hunters, they are neglected target groups for collection of ticks. They have a major potential as receivers of information and as collectors of specimens.

PP 008

Ticks and tick-borne pathogens in Austria 2024

A. M. Schötta[1], *J. Reichl*[1], *K. Bakran-Lebl*[1], *B. Seebacher*[1], *S. Falk*[1], *G. G. Duscher*[2]
L. Winkelmayer[2], *A. Nigsch*[3], *M. Markowicz*[1]
[1]AGES - Austrian Agency for Health and Food Safety, Department for Vector-Borne Diseases, Vienna, Austria
[2]AGES - Austrian Agency for Health and Food Safety, Institute for Veterinary Disease Control, Mödling, Austria
[3]AGES - Austrian Agency for Health and Food Safety, Institute for Veterinary Disease Control, Innsbruck, Austria

In the beginning of 2024, a national tick surveillance programme was established in Austria. The aim of this project is to monitor tick occurrence and composition in Austria and which pathogens they carry.

Citizen scientists are invited to send in ticks from all over Austria but also pictures of suspected *Hyalomma* ticks. The received ticks are morphologically identified and investigated for the presence of tick-borne pathogens such as *Borrelia burgdorferi* sensu lato (Bbsl), *Rickettsia* spp., *Anaplasma phagocytophilum*, *Spiroplasma ixodetis*, *Borrelia miyamotoi* and *Neoehrlichia mikurensis*. In the case of *Hyalomma* ticks, the screening is done for *Rickettsia* spp. and Crimean-Congo haemorrhagic fever virus (CCHFV).

So far, more than 1,200 ticks were investigated. Most ticks belonged to the genus *Ixodes* (84.8%) followed by *Dermacentor* (12.8%), *Haemaphysalis* (1.7%) and other tick species such as *Argas* and *Hyalomma* (0.7%). We confirmed the presence of *Hyalomma* ticks in 11 cases and were able to detect a new way of introduction of this species to Austria – namely tourists.

Overall, 21.3% of ticks were positive for Bbsl. When split into the genera, *Ixodes* was the genus with most positive ticks (24.9%), followed by *Dermacentor* (1.9%). None of the *Haemaphysalis* ticks (n = 21) was positive for Bbsl. The provided *Hyalomma marginatum* ticks (n = 8) were all negative for CCHFV but three (37.5%) were positive for *R. aeschlimannii*. The extended pathogen screening will start beginning of 2025 during another project.

Monitoring the vector landscape by a nation-wide surveillance programme can aid as early detection system when it comes to new exotic diseases or formation of hot spots in certain areas. Knowledge of both endemic and imported tick vectors, along with their associated pathogens, holds significant epidemiological relevance.

PP 009

Community-Driven Tick Collection – a study on the distribution of different tick species from multiple domestic hosts in Mecklenburg-Western Pomerania and Schleswig-Holstein

S. Fischer[1], *S. Drewes*[1], *C. Silaghi*[1]
[1]Friedrich-Loeffler Institut, Institut für Infektionsmedizin, Greifswald, Germany

Background: Citizen science initiatives for vectors such as mosquitoes have extended our understanding of ecological phenomena by harnessing the collective efforts of community members. Therefore, we initiated such a citizen science study with regard to tick species distribution to show which tick species are active, and whether invasive tick species are entering the north of Germany.

Method: The survey was conducted in cooperation with veterinary clinics and veterinary practices across Mecklenburg-Western Pomerania (MV, n=40) and Schleswig-Holstein (SH, n=20) who distributed information flyers and hung up a poster with all the necessary information. Thus, pet owners could become aware of the initiative and sent ticks that were removed or collected from their pets to our laboratory. Ticks are then identified to species level and were statistically evaluated regarding the provided meta data (origin, date, host e.g.).

Results: In 2023, we received over 900 ticks from MV, and until the writing of this abstract, ~2,200 ticks from MV and SH in 2024. So far, morphological species identification revealed: *Ixodes ricinus, Ixodes hexagonus* and *Dermacentor reticulatus*. The majority of obtained ticks were adult and to some extend nymphs from the *Ixodes* spp. The main hosts of the ticks were dogs (~38%), cats (~36%), horses (~2%), but also humans (~1.6%) and for ~20% the host was not specified. The main aim of this study was to dive into the distribution of *D. reticulatus* in northern Germany. Here we could confirm, that it is widely distributed throughout MV. For SH we have only limited information on this species until this Abstract. Further statistical analysis and pathogen screening are planned to evaluate this dataset.

Conclusion: The initiative provided valuable insights into the distribution and diversity of ticks in MV and SH. The results of this study demonstrate the efficacy of community-driven tick collection as a tool to monitor ticks and tick pathogens.

PP 010

New findings of *Hyalomma marginatum* and *Ixodes frontalis* in Slovakia

M. Kazimírová[1]*, Y. M. Didyk*[1,2]*, L. Jancík*[3]*, O. Zhovnerchuk*[1,2]*, B. Mangová*[1]*, V. Rusňáková Tarageľová*[1]
[1]Slovak Academy of Sciences, Institute of Zoology, Department of Medical Zoology, Bratislava, Slovakia
[2]Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine
[3]Keblianska 374/38, Streženice, Slovakia

Ticks can be transported over long distances by migrating birds. As a consequence, immature stages of non-native ticks such as species of the *Hyalomma marginatum* complex and *Ixodes frontalis* have been introduced by migratory birds to several European countries, including Slovakia. Under favourable climatic conditions, these ticks can moult to the adult stage, overwinter, or even establish local populations in Central Europe. In the past, only one questing adult *H. marginatum* female was found in south-western Slovakia in 1981, and one questing male and four nymphs of *I. frontalis* were collected in south-eastern and central Slovakia in 2011-2012. In July 2022, one questing *Hyalomma* female, climbing cloths, was collected in district Púchov (western Slovakia). The tick was morphologically identified as *H. marginatum*. One semi-engorged nymph of *I. frontalis* was collected from vegetation in the Botanical Garden in Bratislava (south-western Slovakia) in February 2024. Morphological identification of both species was confirmed molecularly by PCR targeting specific fragments of the COI and 16S genes. DNA isolated from the ticks was screened for presence of *B. burgdorferi* s.l. complex (only *I. frontalis*), *Rickettsia* sp. and *Babesia* sp. by PCR targeting fragments of the 23S rRNA gene, sca4 and 18S rRNA gene, respectively, all with negative results. The current findings represent the second record of a *H. marginatum* adult and the third record of *I. frontalis* in Slovakia. Although the current risk of introduction of pathogens carried by these ticks to Slovakia is relatively low, continuous monitoring of their occurrence is necessary.

Acknowledgements. The research was funded by grant APVV-22-0372.

PP 011

Emergence of *Hyalomma* ticks in Poland: the contribution of citizen science to tick surveillance

W. Romanek[1], *D. Wezyk*[1], *W. Malaszewicz*[1], *D. Dwużnik-Szarek*[1], *M. AlSarraf*[1], *A. Myczka*[1]
A. Bajer[1]
[1]University of Warsaw, Department of Eco-Epidemiology of Parasitic Diseases, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, Warsaw, Poland

Hyalomma ticks play a crucial role as vectors for the Crimean-Congo haemorrhagic fever virus (CCHFV) and other pathogens. As immatures, they are often transported to temperate regions of Europe from Africa, the Middle East, and Mediterranean areas via migratory birds. The emergence of adult ticks has been documented in numerous countries where they were previously considered non-endemic.

This study aimed to monitor the potential occurrence of *Hyalomma* ticks in Poland using citizen science project. For this purpose, a dedicated website was created through which volunteers could submit photos of unusual ticks. Thanks to an intensive (social)media campaign, the project website attracted tens of thousands of visitors. Between mid-March and mid-November 2024, more than 500 on-line submissions containing tick photos were received, 10 of which were identified as *Hyalomma* ticks.

Additionally, we received 65 parcels containing ticks, including three *Hyalomma* ticks (of 10 on-line submissions). One of these *Hyalomma* was found on a horse, while the other two were not attached to a host and were noticed on the ground.

Amplification of the partial mitochondrial cytochrome C oxidase subunit I gene was successfully performed for all three specimens. Sequencing confirmed the morphological identification for all three ticks. Two of the identified specimens were morphologically and molecularly characterized as *Hyalomma rufipes*, while one was identified as *Hyalomma marginatum.*

Citizen science enabled the identification of 10 *Hyalomma* tick specimens from multiple regions in Poland, marking the first confirmed presence of this genus in the country.

Acknowledgements: The study was funded by the National Science Centre, OPUS grant no. 2022/47/B/NZ6/01610.

PP 012

The Tick Cell Biobank – application of tick cell lines in tick mitogenomic studies

C. Hartley[1], *Y. Suliman*[1], *J. J. Khoo*[1], *A. Darby*[1], *B. Makepeace*[1], *L. Bell-Sakyi*[1]
[1]University of Liverpool, Infection Biology & Microbiomes, Liverpool, United Kingdom

The Tick Cell Biobank (TCB) at the University of Liverpool is the world's only dedicated culture collection for cell lines derived from ticks and other arthropods. We generate, store and supply tick and insect cell lines and training to recipients worldwide, thereby underpinning many aspects of research on ticks, tick-borne diseases and arthropods in general. We also take advantage of the cell line collection to make an in-house contribution to the global efforts in these research areas. For example, we have recently generated complete mitochondrial genome sequences from nine of the tick cell lines held in the TCB, representative of four economically important ixodid genera. These include *Amblyomma variegatum* (AVL/CTVM17), *Hyalomma anatolicum* (HAE/CTVM8 and HAE/CTVM9), *Ixodes ricinus* (IRE/LULS55), *Ixodes scapularis* (ISE6), *Rhipicephalus appendiculatus* (RAN/CTVM3), *Rhipicephalus microplus* (BME/CTVM2 and BME/CTVM6) and *Rhipicephalus sanguineus* (RSE/PILS35). We investigated the phylogenetic relationships of these cell line-derived mitogenomes within the landscape of over 230 publicly available mitogenomes using the concatenation of eight protein-coding genes. Our findings corroborate the taxonomic placement of the selected cell lines and reliably place *A. variegatum* as sister to *Amblyomma hebraeum*. These and many other ixodid and argasid tick cell lines are available from the parent TCB in Liverpool and our TCB Outposts in Malaysia (TIDREC, Universiti Malaya) and Brazil (Fiocruz, Rio de Janeiro), subject to Material Transfer Agreements.

For further information contact tickcellbiobankenquiries@liverpool.ac.uk or visit our website at https://www.liverpool.ac.uk/research/facilities/tick-cell-biobank/.

PP 013

The current state of tick genomics

K. Dillon[1], *J. Frederick*[2,3], *I. Ronai*[4,5], *T. Glenn*[2,1,3]
[1]University of Georgia, Institute of Bioinformatics, Athens, GA, United States
[2]University of Georgia, Department of Environmental Health Science, Athens, GA, United States
[3]University of Georgia, Odum School of Ecology, Athens, GA, United States
[4]Harvard University, Department of Organismic and Evolutionary Biology, Cambridge, MA, United States
[5]Howard Hughes Medical Institute, Chevy Chase, MD, United States

Although more than 900 species of ticks have been described, genome assemblies are available for less than 3% of them. Major challenges for generating genome assemblies of ticks are due to their unique biology, including: a small body size which provides a low amount of DNA, large genome size (typically 2-3 Gbp), high amounts of DNA contamination (from their microbiota and host bloodmeals), and a high proportion of novel transposable elements. Recent advances in genome sequencing technologies (such as, ultra-low DNA input and long-read sequencing) have led to an increasing number of tick genomes, often based on sequences from single individuals, and enabled the generation of high-quality tick genome assemblies. We use a consistent set of bioinformatic approaches to characterize and assess the 56 genome assemblies that are publicly available for 20 tick species within the National Center for Biotechnology Information (NCBI) database as of October 2024. By directly comparing the assemblies, even though most are fragmented into thousands of pieces, we find 25 assemblies have more than 90% of the expected conserved genes. We also summarize how developments in genome sequencing and assembly software have dramatically improved tick assemblies from 2008 to 2024. Finally, we discuss ongoing challenges and future directions for tick genome sequencing and assembly. Tick genome assemblies are critical biological resources that are leveraged to advance tick research, including a better understanding of the molecular basis of tick-host-pathogen interactions and pesticide resistance, alongside identifying potential vaccine targets.

PP 014

EZeSA: Microbiome of ticks in Saxony-Anhalt, Germany

J. Schmidt-Chanasit[1], *G. Geginat*[2], *I. Traut*[3], *R. Luehken*[1], *D. Cadar*[1], *A. Katsounas*[2,3]
[1]Berhard-Nocht Institute for Tropical Medicine, Department of Arbovirology and Entomology, Hamburg, Germany
[2]University Hospital Magdeburg, Medical Microbiology and Hospital Hygiene, Magdeburg, Germany
[3]UniversityHospital Knappschaftskrankenhaus Bochum, Division Infectious Diseases and Critical Care, Bochum, Germany

Background: The prevalence of tick-borne diseases (TBD) is on the rise in Germany, largely due to the combined effects of climate change and the globalization. In response to this growing public health concern, the EZeSA project, which commenced in 2019, is conducting a comprehensive investigation into the spectrum of tick-borne pathogens in Saxony-Anhalt (SA). The objective is to gain a deeper understanding of the associated risks for forestry workers (FW).

Methods: In the initial phase of the study (2019–2020), FW provided ticks that had been collected from their bodies. A total of 580 ticks were collected, identified to species, and the microbiome characterised by metagenomic next-generation sequencing (mNGS). The second phase of the study, which will continue until 2025, will include the analysis of antibodies against specific pathogens from dried blood samples from FW in order to detect exposure to tick-borne pathogens, as well as a systematic interview.

Results: Of the 580 tick specimens analysed, approximately 60% were identified as *Dermacentor reticulatus*, while the remainder were identified as *Ixodes ricinus*, with 80% being nymphs. A total of 5-6 ticks were pooled for mNGS, and 1.3 billion reads were analysed to identify the presence of pathogens. Notably, no *Borrelia* spp. could be detected in the material analysed thus far. The mNGS result was corroborated by a conventional *Borrelia*-specific PCR. Nevertheless, the presence of *Anaplasma* spp. and *Ehrlichia* spp. was confirmed by mNGS in the ticks. Furthermore, novel viruses belonging to the *Flaviviridae* and *Nairoviridae* families were identified through mNGS. **Conclusions:** This ongoing project investigates the prevalence of *Dermacentor reticulatus* and *Ixodes ricinus* on FW in SA and their role as vectors for zoonotic pathogens. Findings will inform public health strategies and risk assessments, emphasizing the need for continuous monitoring of TBD.

Funded by SA-Ministry of Science/Energy/Climate/Environment

PP 015

Update on tick-borne pathogens and new insights into the microbiota of hard ticks in Portugal

L. Moerbeck[1], *J. Lucas*[1], *R. Parreira*[1], *R. Velez*[1], *G. Seixas*[1], *A. S. Santos*[2], *A. Domingos*[1]
S. Antunes[1]
[1]Instituto de Higiene e Medicina Tropical, Vector-borne Diseases group, Lisboa, Portugal
[2]Instituto Nacional de Saúde Doutor Ricardo Jorge, Centro de Estudos de Vectores e Doenças Infecciosas Dr. Francisco Cambournac, Águas de Moura, Portugal

Ticks are blood-feeding ectoparasites hosting diverse microorganisms, including symbiotic, commensal, and pathogenic species, influencing their physiology and pathogen transmission. The aim of this study is twofold: firstly, to investigate tick-borne pathogen (TBP) prevalence in ticks from mainland Portugal; and secondly, to assess the impact of infection on *Rhipicephalus bursa* microbiota tissues, as well as the influence of collection site on the whole microbiota of *Ixodes ricinus*.

To achieve the first aim, questing ticks were collected from five ecological areas between 2019 and 2021 using the dragging method. Following morphological identification, DNA/RNA was extracted to screen for TBPs, including *Babesia spp.*, *Theileria spp.*, *Rickettsia spp.*, *Anaplasma* spp., *Ehrlichia* spp., *Neoehrlichia mikurensis*, *Coxiella burnetii*. Regarding the second aim, microbiota analysis was conducted using Illumina MiSeq sequencing of the 16S *rRNA* gene (V3-V4 regions) in field-collected *I. ricinus* females and laboratory-reared *R. bursa* females (uninfected and infected with *Babesia ovis*). The Minimum Entropy Decomposition (MED) algorithm was used to identify operational taxonomic units (OTUs), further assignments were analyzed with QIIME software package.

TBP screening detected *Babesia bigemina*, *Theileria sp.*, *Rickettsia helvetica*, *R. monacensis*, *R. massiliae*, *R. slovaca*, and *Candidatus Rickettsia rioja*. Microbiota analysis of *R. bursa* revealed 1,525 OTUs, with *Sphingomonas* dominating uninfected tissues but reduced in infected ones. In *I. ricinus*, 804 OTUs were identified, from samples collected in Tunisia showing greater microbiota diversity compared to those collected from the Iberian Peninsula. *Midichloria mitochondrii* was consistently present across all ticks. The present study updates knowledge on TBP prevalences in questing ticks from mainland Portugal and provides novel insights into the intricate interactions between ticks, pathogens, and their microbiota.

PP 016

Comparative bacterial communities and population genetics between bisexual and parthenogenetic *Haemaphysalis longicornis* in South Korea

J. Kim[1], *S. Hwang*[1], *D. Kim*[1]
[1]Kyungpook National University, Sangju-si, South Korea

The Asian longhorned tick, *Haemaphysalis longicornis*, is an ectoparasite known to transmit various pathogens, including the severe fever with thrombocytopenia syndrome virus, to both humans and animals. Wild populations of *H. longicornis* exhibit both bisexual and parthenogenetic reproductive strategies. This study aimed to investigate the bacterial communities and genetic differentiation between bisexual and parthenogenetic populations of *H. longicornis* collected from 12 cities across the Republic of Korea: Sokcho (SC), Chuncheon (CC), Ganghwa (GH), Samcheok (SCH), Sangju (SJ), Boryeong (BR), Ulsan (US), Gochang (GC), Jinju (JNJ), Jindo (JD), Jeju (JJ), and Seogwipo (SG). Parthenogenetic and bisexual individuals coexisted in varying proportions depending on the region, with parthenogenetic individuals predominating in the northeastern regions (CC, SJ, US, SC, GH, SCH, and JNJ), and bisexual individuals more abundant in the southwestern regions (JD, SG, JJ, BR, and GC). Genetic analysis using concatenated sequences of cytochrome c oxidase subunit I (*COI*) and cytochrome B (*cytB*) genes revealed two major haplotypes associated with the two reproductive modes. Phylogenetic analysis indicated that female ticks clustered according to their reproductive system rather than geographical location. Additionally, bacterial community analysis revealed that the genus *Coxiella* predominated in both reproductive systems of *H. longicornis*, while the genus *Rickettsia* was more prevalent in bisexual individuals compared to parthenogenetic ones. These findings suggest that bisexual and parthenogenetic populations of *H. longicornis* co-exist in the Republic of Korea, with bacterial communities such as *Coxiella* and *Rickettsia* differing in prevalence depending on the reproductive system. This study enhances our understanding of the reproductive strategies and vectorial capacity of *H. longicornis*.

PP 017

Identification of ion transport peptide-like (ITPL) signalling in the tick *Ixodes ricinus*

V. Klöcklerová[1], *D. Žitňan*[1]
[1]Slovak Academy of Sciences, Institute of Zoology, Dept. of Molecular Physiology, Bratislava, Slovakia

Ticks are hematophagous ectoparasites whose lifecycle relies on maintaining water homeostasis. The primary osmoregulatory organs - salivary glands and hindgut – are regulated by neurons of central nervous system (CNS) producing neuropeptides, which act as neuromodulators, neurotransmitters, or hormones. Ion transport peptide (ITP) and an ITP-like (ITPL) are alternatively spliced neuropeptides, in insects involved in many physiological processes including regulation of development and water homeostasis. In this study we focused on ITP and ITPL of the tick *Ixodes ricinus*. Based on molecular cloning and rapid amplification of cDNA ends, the *itp* gene of *I. ricinus* encodes two alternatively spliced ITPLs – ITPL1 and ITPL2 - but no ITP. According to qPCR, expression of both ITPLs is restricted to synganglion. Further in situ hybridisation with ITPL1- and ITPL2-specific probes revealed the transcripts within synganglion are differentially expressed. Both transcripts are present in a huge pair of neurons in post-oesophageal region of the opisthosomal lobe. ITPL1 is further expressed in 3 pairs of heavily stained dorso-lateral protocerebral neurons, while expression of ITPL2 was detected in limited number of interneurons of the protocerebral lobe. We identified and completed sequences of two putative ITP/L receptors – homologs of BNGR-A2 and BNGR-A24 of the silkworm *Bombyx mori*. Both putative receptors are expressed in synganglion, female gonads and gut, suggesting ITPL's action outside the synganglion.

Our results confirm the presence of ITPL signalling in the tick *I. ricinus*. We also demonstrate the absence of the insect major regulator of water homeostasis, ITP, in *I. ricinus* ticks.

Acknowledgement: This study was supported by SAS PostdokGrant No. APD0086.

PP 018

Enzymes beyond tick midgut hemolysis

T. Kozelková[1,2], *D. Sojka*[1], *P. Kopacek*[1]
[1]Biology Centre CAS, Institute of Parasitology, České Budějovice, Czech Republic
[2]University of South Bohemia, Department of Chemistry, České Budějovice, Czech Republic

The host blood serves as the sole source of energy and nutrients for all ticks. The first step in blood digestion is the release of hemoglobin from host red blood cells (RBCs) by hemolysis. While the subsequent molecular mechanism of hemoglobin digestion is well described, the mechanism underlying hemolysis and associated hemolytic activity remain unexplored in the model tick *Ixodes ricinus*.

In contrast to mosquitoes, lysis of host erythrocytes in ticks appears to be an enzymatic process mediated by specific hemolytic enzymes, termed hemolysins. Previous studies have shown that hemolysis in various tick species was destroyed by trypsin treatment or after boiling. Despite these findings, the exact identification of hemolysins in ticks has not yet been unequivocally established. In principle two types of enzymes can be considered: phospholipases zipping-off the double-layered phospholipid membranes of erythrocytes or proteases destroying protein components of the outer RBC membrane, leading to its destruction.

Both concepts have already been evaluated. While in tick saliva a possible role of calcium-dependent phospholipase A2 (PLA 3) was suggested its role was excluded in hemolytic activity in *Ixodes dammini* midgut due to the no inhibition by EDTA. On the other hand, RBCs lysis in the midgut lumen of the tick *Haemaphysalis longicornis* has been reported to be mediated by two cubilin-related serine proteases. However, none of above mentioned works led to a satisfactory identification of the tick hemolysin(s).

Hemolytic assays have been established and hemolytic activity has been assessed in midguts during the adult tick females feeding. Additionally, several lipase or protease inhibitors have been tested. These results revealed that both, lipases and serine proteases act as hemolysins in the hard tick *I. ricinus*.

PP 019

Microscopic anatomy of the midgut of *Ixodes ricinus* nymphs in different feeding stages

V. Urbanova[1,2], *D. Sojka*[1,2], *M. Vancova*[1,3], *F. Kitzberger*[1,3], *J. Tyc*[1], *T. Bily*[1], *T. Kozelková*[1,3]
P. Kopacek[1]
[1]Biology Centre, Institute of Parasitology, Ceske Budejovice, Czech Republic
[2]Slovak Academy of Sciences, Institute of Zoology, Bratislava, Slovakia
[3]University of South Bohemia, Faculty of Sciences, Ceske Budejovice, Czech Republic

Ticks are globally distributed ectoparasites that depend on the blood of their host to obtain essential nutrients. Blood digestion takes place in the digestive cells of the midgut, while the midgut lumen serves primarily as a food reservoir. This mechanism allows juvenile ticks to survive longer intervals between feedings and helps adult females to produce large numbers of eggs.

In our study, we used immunofluorescence and specific TEM immunolabeling on cryosections to investigate the structural changes in the midgut of nymphs of *I. ricinus* from the unfed stage to four weeks post-detachment. We have focused on the dynamics and distribution of lipid droplets (LDs), the organelles that are crucial for the management of lipid reserves. Next, we examined the distribution of hemoglobin (Hb) in the midgut of nymphs at different feeding stages, as ticks depend on host-derived hemoglobin due to their inability to synthesize heme.

To examine LD distribution throughout the whole gut nymphs, we used LD540 dye staining. For more precise ultrastructural identification, we performed immunogold labeling with the specific antibody on Tokuyasu cryosections of the midgut of nymphs at different stages. In addition, electron microscopy with immunogold labeling was used to observe the dynamics and distribution of Hb vesicles at the same time points as in LD immunodetection. In parallel, we performed serial block-face scanning electron microscopy (SBF-SEM) followed by 3D image reconstruction and TEM tomography to further analyze the midgut of nymphs 48 hours of feeding.

Our results show the distribution of hemoglobin, albumin and lipid droplets in the midgut cells before, during and after feeding. These components play a crucial role in the metamorphosis of the nymphs and the subsequent development of the adult male and female ticks.

Acknowledgement: BC CAS core fac. LEM supported by MEYS CR (LM2023050 Czech-BioImaging to M.V.) and the Czech Science Foundation (grant No. 22-12648J to P.K.)

PP 020

Molecular screening of the physiological roles of neuropeptides and GPCRs in attachment and salivary secretion from *Haemaphysalis longicornis*

S. Hwang[1], *J. Kim*[1], *D. Kim*[1]
[1]Kyungpook National University (South Korea), Vector Entomology, Sangju si, South Korea

Tick salivary secretion is known to be regulated by various neuropeptides and their receptors (G protein-coupled receptors, GPCRs) via the peptidergic axonal projection from the synganglion. During the slow phase (4-5 days after blood feeding) of tick blood feeding, two dopamine receptors, the dopamine receptor (D1) and the invertebrate specific D1-like dopamine receptor (InvD1L), orchestrate the dynamic response of type III acini for salivation, which is controlled by inward fluid transport and expulsion of primary saliva, respectively. However, the physiological role of other neuropeptides and GPCRs during tick feeding remains unclear. To investigate the physiological role of neuropeptides including sNPF, NPF, Elevenin, and SIFamide and GPCRs including D1 and InvD1L during different phases of tick blood-feeding, synganglion and salivary glands were prepared from unfed, 3, 18, 60, and 96 h post blood feeding, as well as engorged *Haemaphysalis longicornis* to profile differentially expressed transcripts. In addition, the physiological functions of neuropeptides and GPCRs were examined after the knockdown of target genes using RNAi. The results showed extended tick attachment and lower salivary secretion rate compared to control ticks. The proteomic analysis may provide further insights into the physiological role of neuropeptides and GPCRs during tick feeding.

PP 021

Optimization of a novel artificial feeding system for the lone star tick, *Amblyomma americanum*

M. Burch[1,2], *D. Bente*[1,2]
[1]University of Texas Medical Branch, Department of Microbiology & Immunology, Galveston, United States
[2]Galveston Nation Laboratory, Galveston, United States

Lone star ticks (*Amblyomma americanum*), widespread in the eastern United States, are aggressive and important vectors of viral and bacterial pathogens, including Heartland and Bourbon virus and *Ehrlichia* and *Rickettsia spp*. To study pathogen transmission dynamics, artificial feeding systems provide a controlled environment to reduce experimental variability and minimize animal use in vector research. Unlike traditional well-plate setups, an inverted feeding system encourages tick migration and may enhance feeding rates. In this study, we optimized an inverted artificial feeding system developed by the U.S. Department of Agriculture (USDA) for use with lone star ticks. The USDA system has considerable potential for optimization, including individual temperature control for feeding units and a closed-loop system for adding a peristaltic pump to circulate blood. With our current project, we evaluated various blood meal additives, membranes, and attractants to improve feeding. Our results show that siliconized Goldbeater"s skin with autoclaved hair enhanced tick feeding, resulting in adult female attachment (80%), partial and full engorgement (73.3%), and oviposition (36.4%). This study established feeding protocols that will support future pathogen transmission research and may be adapted for other medically significant tick species.

PP 022

Results from eight years of surveillance of ticks and tick-borne encephalitis virus in southern Norway

A. P. Cotes Perdomo[1,2], *A. Soleng*[1], *A. Jenkins*[2], *K. Alfsnes*[3], *H. Renssen*[2], *R. Vikse*[1]
Å. K. Andreassen[1]
[1]Norwegian Institute of Public Health, Virology, Oslo, Norway
[2]University of South-Eastern Norway, Department of Natural Sciences and Environmental Health, Bø, Norway
[3]Norwegian Institute of Public Health, Bacteriology, Oslo, Norway

Macro- and microclimatic conditions affect in a plethora of ways the questing behavior of ticks, their survival, and reproduction success. It is expected then that the phenology of ticks and tick-borne pathogens will vary following weather conditions and climate. In Norway, Vest Adger used to be the focus of tick-borne encephalitis (TBEV), but despite the number of cases has duplicated there in the last years, the foci are located more to the west in Agder and Vestfold counties. Surveillance of TBEV prevalence in ticks from Mandal (Vest Agder) has been carried out from 2009 until 2022. In parallel, monthly assessments of tick density/activity have been carried out since 2017 until present in Mandal and Horten (Vestfold). During this period in Mandal, TBEV's average prevalence has been 0.35% in nymphs and 6% for adults, presenting its peaks in summer or early autumn months, being undetectable in ticks after years with strong variations in temperature and precipitation regimes. The prevalence of TBEV and the density of questing ticks vary independently. Concerning the variation in the density of questing ticks, an increase in the number of larvae, nymphs, and adult ticks has been constant during these eight years in Mandal and Horten, without a clear association with meteorological variables such as temperature and humidity. More analyses are being done for a better understanding of this trend, including contrasting data on ungulates' and other mammals' abundance, as well as that of birds. At the same time, our data is being fitted within different climate change scenarios.

PP 023

Establishing multicellular blood-brain barrier infection models for antiviral drug discovery

M. Zuniga[1]*, A. Herrmann*[1]*, M. Rizzato*[1]*, J. J. Bugert*[2]*, R. Brack-Werner*[1]
[1]Helmholtz Zentrum München, Virology, Oberschleissheim, Germany
[2]Bundeswehr Institute of Microbiology, Virology, Munich, Germany

Neurotropic viruses, like tick-borne encephalitis virus (TBEV), are capable of infecting cells of the central nervous system (CNS) that may lead to encephalitis. These viruses often gain access to the CNS through routes involving the blood-brain barrier (BBB), a critical cellular unit protecting the brain parenchyma from toxins and pathogens in circulation. The protection provided by the BBB is possible through the crosstalk and the unique physical characteristics of the cells forming this barrier. However, the strong barrier function of the BBB may also represent a severe obstacle to the passage of antiviral agents to the brain. To discover or repurpose compounds capable of targeting neurotropic viruses, like TBEV, we are establishing *in vitro* models of the BBB with human brain microvascular endothelial cells (HBMEC), pericytes (HBMVP), and astrocytes differentiated from an immortalized neural stem cell line (HNSC.100). Through cytometric and immunofluorescence techniques, we identified unique cellular markers for HBMEC, HBMVP, and astrocytes, that allow us to distinguish between the different cell types. Utilizing a fluorometric (permeability) assay, co-cultures with HBMVP decrease the permeability of HBMEC monolayers, demonstrating the importance of cellular crosstalk in promoting BBB tightness. Initial *in vitro* infection experiments reveal TBEV crosses a co-culture of HBMEC and HBMVP to infect target cells with no evident effect on barrier integrity. These *in vitro* BBB models can serve as predictive screening tools for the passage of compounds capable of targeting viruses in the brain. This will help reduce animal experimentation and expedite the development of new therapies for virus-induced diseases of the nervous system. Along with compound screening, the models offer the opportunity to investigate the mechanisms employed by neurotropic viruses to breach the BBB and the cellular responses involved in causing encephalitis.

PP 024

Prevalence and risk factors of tick-borne encephalitis virus in dogs in Lithuania

A. Stankevicius[1], A. Pautienius[1], E. Simkute[1], J. Grigas[1], A. Paulauskas[2], J. Radzijevskaja[2]
[1]Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Vytautas Magnus University, Research Institute of Natural and Technological Sciences, Kaunas, Lithuania

Tick-borne encephalitis (TBE) is recognized as the most important zoonotic tick-borne viral disease in Europe. Causative agent of the disease is typically transmitted to human beings through bites of tick-borne encephalitis virus (TBEV)-infected ticks. However, an increasing number of case reports of TBE infection in dogs indicate that the virus might be an important tick-borne pathogen in dogs, especially in endemic areas. Therefore, the aim of the present study was to investigate the prevalence of TBEV RNA and TBEV-specific antibodies in serum samples of dogs living in a highly endemic region of Lithuania and to evaluate the main risk factors associated with the severe cases of TBE.

The serum samples ($n = 473$) of dogs were randomly collected in two veterinary clinics in central Lithuania and analysed by commercially available ELISA kits and nested RT-PCR using primers targeting NCR5 region. To confirm TBEV propagation, Neuro-2a, Vero and MARC-145 cells were transfected with suspensions prepared from positive sera and viral replication in cells was confirmed via RT-PCR.

Observed neurological symptoms in 31.8% (CI 95% 22.3-42.6) and a death rate of 18.2% (CI 95% 10.8-27-8) of PCR-positive dogs implies that TBE is a clinically important disease in dogs in endemic countries such as Lithuania. TBEV-specific antibodies were detected in 21.6% (CI 95% 17.9-25.6) of randomly collected dog blood samples and in 32.1% (CI 95% 15.9-52.4) of dogs with neurological symptoms. Males were more likely to develop neurological symptoms ($p = 0.0084$), while older dogs ($p = 0.0032$), dogs with neurological symptoms ($p = 0.003$) and the presence of TBEV-specific antibodies ($p = 0.024$) were more likely to experience worse outcomes of the disease. The results of the present study demonstrate that TBEV is a common and clinically important pathogen in dogs in Lithuania.

Funding: This research was funded by the Research Council of Lithuania (Grant No. S-MIP -23/19).

PP 025

Studies on the co-localisation of TBE-Virus and *Borrelia* species at selected TBEV-foci in Baden-Wuerttemberg

R. Vogt[1], *K. Fachet-Lehmann*[1], *A. Lindau*[1], *M. Grünke*[1], *S. Laib*[1], *A. Straube*[1], *J. Oberle*[1]
U. Mackenstedt[1]
[1]University of Hohenheim, Department of Parasitology, Stuttgart, Germany

In Central Europe, *Ixodes ricinus* ticks are vectors for a wide range of pathogens, including the tick-borne encephalitis virus (TBEV) and *Borrelia burgdorferi* s.l. Both pathogens are of public health importance. In Germany, the TBEV is primarily represented by a genetically diverse subgroup of the European subtype, while *Borrelia* has an even greater genetic diversity and comprises at least eight species that can infect humans. Co-infections of ticks with multiple pathogens, such as TBEV and various *Borrelia* species, have been documented, but the ecological and molecular interactions between these pathogens remain unclear.

This study investigates the potential influence of *Borrelia* on the prevalence and spatial distribution of TBEV within tick populations. Specifically, we examine the co-infection dynamics between various TBEV strains and *Borrelia* species within TBEV microfoci in the district of Ravensburg, a region experiencing a sharp increase in human TBE cases since 2017. By analyzing TBEV foci and nearby TBEV-free sites, both local and in the metropolitan area of Stuttgart as controls, we aimed to address whether specific *Borrelia* species are associated with TBEV foci and if co-infections with *Borrelia* may influence the restricted distribution patterns observed for TBEV in the region.

A total of 4,801 *Ixodes ricinus* ticks were collected from the different sites and subsequently screened for the presence of TBEV and *Borrelia*. Co-infections within individual ticks carrying both pathogens were identified, offering important insights into the potential interactions between these pathogens within tick populations. The preliminary results indicate that TBEV and *Borrelia* infections can occur simultaneously in individual ticks and are a factor of considerable importance for epidemiological modeling and the development of targeted control strategies.

PP 026

Tick-borne encephalitis virus NS1 disrupts the integrity of endothelial monolayer

M. Čížková[1,2]*, M. Davídková*[1]*, M. Dvořáková*[1]*, H. Sehadová*[2,3]*, V. Prančlová*[1,2]*, E. Kotounova*[1,2]
M. Palus[1,4]
[1]Biology Centre CAS, Laboratory of Arbovirology, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
[3]Biology Centre CAS, Laboratory of Microscopy and Histology, Ceske Budejovice, Czech Republic
[4]Veterinary Research Institute, Department of Virology, Brno, Czech Republic

Tick-borne encephalitis virus (TBEV), a member of the genus *Orthoflavivirus* in the family Flaviviridae, is the origin of a devastating neurological disease in Europe and northeast Asia. TBEV attacks central nervous system (CNS), but for its infection virus must overcome the blood-brain barrier (BBB), which is responsible for safeguarding the CNS. One of the main components that can play a role in the safeguarding of CNS is endothelial layer and its glycocalyx. TBEV non-structural protein 1 (NS1) is the protein with the potential to compromise the endothelial layer within BBB.

We have studied ability of the NS1 in disruption endothelial monolayer. The experiments were carried out *in vitro* on human primary endothelial cells and integrity of cell monolayer was examined. It was made through Transwell system, which enable us imitate human BBB. In addition, the endothelial glycocalyx components were analysed through immunofluorescence antibody staining (IFA).

Our model demonstrated, that TBEV NS1 is able to disrupt the integrity of endothelial monolayer. Also, through IFA and subsequent mean fluorescence analyse (MFI), we demonstrate that NS1 is also able to alter the endothelial glycocalyx.

These findings underscore the critical role of TBEV NS1 in compromising BBB integrity, enhance our understanding of its neuroinvasive potential, and provide valuable insights for the development of targeted therapeutic strategies for TBEV-associated neurological diseases.

PP 027

Characterisation of tick-borne encephalitis virus from *Ixodes ricinus* ticks from eastern German low mountain ranges

L. M. I. Maas[1], *G. Dobler*[2], *L. Chitimia-Dobler*[2], *A. Obiegala*[1], *M. Pfeffer*[1]
[1]University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany
[2]Bundeswehr Institute of Microbiology, Munich, Germany

Tick-borne encephalitis (TBE) is caused by the tick-borne encephalitis virus (TBEV) and the most important tick-borne human infection of the central nervous system in Europe and Asia. Humans are accidental hosts who can become infected through the bite of an infected tick or by consuming unpasteurised milk from TBE-infected animals. Although the disease is preventable by vaccination, an increase in the incidence of TBE cases and a geographical spread of the disease has been recorded in recent years. In Germany, a steady rise in reported TBE cases has been registered since around 2016, especially in the states of Saxony and Thuringia.

We were interested whether the TBE cases in these states were associated with certain habitats and natural areas. We contacted the state examination offices (Landesuntersuchungsämter) in Saxony and Thuringia in order to forward a specific questionnaire to the state health authorities and thus to reach as many people affected by TBE as possible in order to gain details about the places where their infection was acquired. This proved to be very successful and approx. 60 locations were investigated by tick flagging. In the course of this study, ticks from natural TBE foci were collected in seven different natural areas over a period of two years in the eastern German low mountain ranges by using the flagging method.

About 2,700 ticks were collected and tested for TBEV by real-time RT-PCR. One new TBEV-spot in Thuringia and two new TBEV-spots in Saxony could be identified. The range of the minimal infection rates (MIR) is differing between 0.012% and 0.037%. Our analysis revealed that the isolated TBEV strains belong to the European Subtype (TBEV-Eu). The results of the phylogenetic analyses are still pending.

The financial support by Pfizer Pharma GmbH, Berlin, is greatly acknowledged. We thank Sara Weilage, Christiane Frömel, Pauline Lenz and Caroline Frank for their help during field work.

PP 028

Wild rodents as sentinel hosts for tick-borne encephalitis virus – a study of TBEV prevalence in TBEV foci in Lithuania

A. Pautienius[1], *E. Simkute*[1], *J. Radzijevskaja*[2], *J. Grigas*[1], *M. Sidorenko*[2], *A. Paulauskas*[2]
A. Stankevicius[1]
[1]Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Vytautas Magnus University, Kaunas, Lithuania

Wild rodents are recognized as key reservoir hosts for tick-borne encephalitis virus (TBEV), making them valuable indicators for identifying TBEV foci. This study aimed to evaluate the efficacy of viral RNA detection in rodents for confirming suspected TBEV hotspots. A total of 139 small rodents were captured from various known TBEV-endemic locations across Lithuania. Rodent samples were cultured in murine neuroblastoma Neuro-2a cells to increase viral RNA detectability. TBEV RNA was detected in 74.8% (95% CI: 66.7–81.1) of brain and internal organ mix suspensions. The prevalence rate significantly increased after sample cultivation in Neuro-2a cells. Notably, a strong correlation ($r = 0.88$; $p < 0.05$) was observed between TBEV RNA prevalence after cultivation in cells and the average monthly air temperature during rodent trapping. This study highlights the suitability of wild rodents as sentinel hosts for TBEV surveillance and demonstrates the effectiveness of cell culture methods in amplifying viral load for detection.

PP 029

Quasispecies in tick-borne encephalitis virus – utilising long-read next-generation sequencing to determine population diversity and genomic plasticity in tick-borne flaviviruses

L. Tardy[1], *V. Hönig*[1,2], *J. Kamiš*[1,3], *D. Růžek*[1,2,4], *M. Palus*[1,2]
[1]Biological Center of the ASCR, vvi, Institute of Parasitology, Ceske Budejovice, Czech Republic
[2]Veterinary Research Institute, Brno, Czech Republic
[3]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
[4]Masaryk University, Department of Experimental Biology, Brno, Czech Republic

Quasispecies theory has been widely adapted to study the evolutionary biology of RNA viruses, where large population sizes, small genomes and high rates of error-prone replication give rise to dynamic and heterogeneous viral populations[1]. For members of *Flaviviridae*, a family of single-stranded positive-sense RNA viruses often with wide host-tropisms and complex eco-epidemiological histories, quasispecies may play a large role in maintaining viral adaptability and plasticity as well as genomic robustness over time. A growing body of literature employing quasispecies theory is beginning to unravel the underlying processes and evolutionary dynamics of these viruses, for example through recent work on the mosquito-vectored West Nile viruses and the tick-vectored tick-borne encephalitis virus (TBEV) [2-3].

Utilising TBEV as our primary model, our group are developing protocols which apply long-read next generation sequencing methodologies to study viral quasispecies at the whole-genome level, furthering our ability to understand the effects of distant mutations acting in combination and to better measure viral population diversity at the level of single-infections. We present preliminary findings of quasispecies diversity in TBEV, validation of our experimental and analytical pipelines and discuss how long-read NGS and computationally-driven quasispecies reconstruction can further our study of viral evolution.

REFERENCES

1. Domingo, E., Perales, C. Quasispecies and virus., 2018, Eur Biophys J, 2018, 47, 443-457.
2. Kortenhoeven, C., Joubert, F., Bastos, A.D., Abolnik, C., 2015, Virus genome dynamics under different propagation pressures: reconstruction of whole genome haplotypes of West Nile viruses from NGS data. BMC Genomics, 2015, 118.
3. Helmová, R., Hönig, V., Tykalová, H., Palus, M., Bell-Sakyi, L., Grubhoffer, L., 2020, Tick-Borne Encephalitis Virus Adaptation in Different Host Environments and Existence of Quasispecies. Viruses, 12(8):902.

PP 030

Characterisation of viral-derived DNA (vDNA) forms in *Hyalomma lusitanicum* tick cell line in response to Hazara virus infection

E. Dias[1,2]*, S. Fabi*[3]*, F. Monteiro*[1]*, C. Salata*[3]*, A. Domingos*[1]*, G. Seixas*[1,2]
[1]Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Lisbon, Portugal
[2]Gulbenkian Institute for Molecular Medicine (GIMM), Biochemistry, Lisbon, Portugal
[3]University of Padua, Molecular Medicine, Padua, Italy

The Crimean-Congo hemorrhagic fever virus (CCHFV) and Hazara virus (HAZV) belong to the same serotype and viral family. CCHFV causes Crimean-Congo hemorrhagic fever, a tick-borne disease with a mortality rate of up to 40%. In recent decades, the expansion of its vector's range has led to increased disease spread. Consequently, understanding the molecular mechanisms underlying viral infection and transmission is crucial to combatting this illness. This study aims to provide further insights into virus-vector interactions by using HAZV and tick cell lines as an experimental model.
During viral infection, viral-derived DNA (vDNA) molecules are formed from viral RNA genomes through an endogenous reverse transcriptase activity in the host cell. This mechanism, still underexplored in ticks, is thought to play a key role in establishing persistent infections. The study objectives were to:

1. Characterize vDNA production and the viral progeny at multiple time points post-infection, ranging from 3 to 60 days, with varying multiplicities of infection (MOI) in *Hyalomma* cell lines.

2. Investigate the cellular localization of vDNAs.

3. Assess the impact of AZT, a drug that inhibits vDNA formation, on cell survival, viral replication, and the production of new viral particles.

The data collected indicated that vDNA formation is dependent on HAZV infection, with these molecules detected in both the nucleus and cytoplasm. Furthermore, vDNA formation appears essential for establishing persistent infections, as its inhibition led to increased viral particle production in cells, ultimately resulting in cell death.

This study aims to contribute to a better understanding of the molecular interactions between viruses and their vectors, potentially paving the way for novel therapeutic approaches or preventive strategies.

PP 031

Tick species and TBEV Surveillance in Brønnøy, Northern Norway

A. Saiyara[1,2], *A. P. Cotes Perdomo*[1,3], *R. Vikse*[1], *K. Alfsnes*[4], *H. E. H. Linstedt*[3], *W. Johansen*[2]
A. Soleng[3], *Å. K. Andreassen*[1,3]
[1]Norwegian Institute of Public Health, Virology, Oslo, Norway
[2]Inland Norway University of Applied Sciences, Faculty of Applied Ecology, Agriculture and Biotechnology, Hamar, Norway
[3]Norwegian Institute of Public Health, Pest Control, Oslo, Norway
[4]Norwegian institute of Public Health, Bacteriology, Oslo, Norway

Ticks are widely recognized as significant vectors of pathogens, contributing to the growing prevalence of tick-borne diseases (TBDs). The geographical range of tick species is expanding, with species from Europe and Asia spreading between continents. Ticks from Europe and Asia differ in their feeding behavior, host preference, and ability to transmit pathogens. The most common tick in Europe is Ixodes ricinus, while Ixodes persulcatus is the most common in the Russian part of Asia. Ixodes persulcatus is showing a persistent population in the middle of Finland but has recently been identified in Northern Sweden. This species, more commonly found in Eastern Europe, is commonly infected with all three main genotypes of tick-borne encephalitis virus (TBEV). It also shares sympatric populations with I. ricinus in countries like Russia, Finland, and the Baltics. There is evidence suggesting that I. persulcatus may inhabit the southern part of Northern Norway. Particularly, there is a risk of detection of I. persulcatus in areas close to Brønnøy municipality, which are geographically close to Swedish regions with known I. persulcatus populations. A sample collection of 250 nymphs sampled from May to July in Brønnøy was analyzed by a multiplex PCR for verifying Ixodes strains. All tested ticks were entirely of the I. ricinus strain, and none tested positive for TBEV. However, it is suspected that nymphs and adult ticks collected during June and July may yield TBEV-positive results. However, none of the analyzed nymphs tested positive for TBEV. If I. persulcatus is found in Norway, it would raise important questions about its role as a vector of severe TBDs and the factors driving its spread. Identifying the specific subtypes of Ixodes species is crucial for understanding their epidemiological significance and developing effective surveillance and mitigation strategies to combat the growing threat of TBDs.

PP 032

Epidemiology of Crimean-Congo Hemorrhagic Fever Virus in Tunisia, North Africa

K. Dachraoui[1], *S. Dowall*[1], *O. Jlassi*[1], *Y. Maachach*[1], *M. Holding*[1], *H. Younsi*[1], *R. Hewson*[1]
H. Sprong[1], *E. Zhioua*[1]
[1]Institut Pasteur de Tunis, Tunis, Tunisia

Crimean-Congo hemorrhagic fever virus (CCHFv) is the etiologic agent of CCHF and transmitted mainly through the bite of ixodid ticks. The geographical distribution of the arthropod vector covers most of the Western Mediterranean basin. CCHFv has been detected in *Hyalomma marginatum* in Morocco and in *H. aegyptium* in Algeria providing strong evidence of *Hyalomma spp.* incrimination in viral transmission.

We aimed to perform a sero-epidemiological survey to test for circulation of CCHFv in Tunisia and to assess samples of *Hyalomma spp.* ticks for the presence of viral RNA. Samples from unexplained acute febrile patients (n=181) and slaughter workers (n=38) were collected and analyzed for exposure to CCHFv using serological tests. Of the 181 febrile patients, 5 showed high titers of IgM suggesting a recent exposure to CCHFv. Among 38 slaughter workers, 2 had IgG anti-CCHFv responses yielding a seroprevalence of 5.2%.

A total of 310 *H. dromedarii* ticks were collected from Oryx in the national park located in Southern Tunisia over the period May–June 2014 and evaluated for the presence of viral RNA using real-time RT- PCR. In addition, a total of 1,174 *H. aegyptium* ticks were collected from spur-thighed tortoises captured in Northern Tunisia from March to April 2017. RT-PCR analysis failed to detect the presence of CCHFv RNA, indicating that the analyzed ticks are unlikely to play a major role in the epidemiology of CCHF. A high seroprevalence of CCHFv was reported from camels in Southern Tunisia. During April 2024, more than 500 *H. dromedarii* collected from camels in Southern Tunisia are being analyzed for the presence of viral RNA using real-time RT- PCR.

Our results provide evidence of human exposure to CCHFv in Tunisia. Thus, further studies are recommended to be carried out on livestock, high risk groups of humans, birds, and other tick species for a better understanding of the dynamic transmission of CCHFv in Tunisia and surrounding countries in North Africa.

PP 033

Identifying primary and alternative tick vectors for Crimean-Congo hemorrhagic fever virus: the importance of defining vectorial capacity of ticks for human risk

D. Bente[1,2], *M. Burch*[1,2]
[1]University of Texas Medical Branch, Department of Microbiology & Immunology, Galveston, United States
[2]Galveston Nation Laboratory, Galveston, United States

The geographic range of Crimean-Congo hemorrhagic fever virus (CCHFV) is the most extensive of the medically significant tick-borne viruses. It is the second most widespread of all hemorrhagic fever viruses, after dengue virus. It causes an estimated 10,000-15,000 cases per year with at least 500 deaths. According to the WHO, an estimated 3 billion people are at risk of infection. CCHFV circulates in a tick-vertebrate-tick cycle in nature, with neither animal hosts nor ticks showing any signs of disease. Ixodid ticks of the genus *Hyalomma* are considered the reservoirs and primary vectors. However, the virus has been detected in over 31 tick species. Although *Hyalomma* spp. have been defined as the most essential and primary tick vectors for CCHFV, it is unclear what tick species play what role in other parts of the world. Vectorial capacity is a mathematical equation widely used in mosquito-borne diseases that integrates significant vector biology aspects and transmission dynamics to quantify a vector's ability to spread disease. Vectorial capacity is a crucial metric for determining a tick species' importance as a disease vector, evaluating potential disease emergence and spread, and assessing the effectiveness of vector control measures. Here, we propose how the vectorial capacity of different CCHFV vectors can be evaluated to determine human risk.

PP 034

Tick-borne pathogens in pet and hunting dogs in Kaunas, Lithuania

K. Jankauskaitė[1,2], J. Radzijevskaja[1,2],M. Laukuté[1,2], B. Karveliené[2], A. Paulauskas[2], Z. Gintaras[2]
J. Radzijevskaja[1]
[1]Vytautas Magnus University, Research Institute of Natural and Technological Sciences, Kaunas, Lithuania
[2]Lithuanian University of Health Sciences, Faculty of Veterinary Medicine, Kaunas, Lithuania

Tick-borne diseases pose a significant threat to animal and human health in the Baltic region, including Lithuania, due to the temperate climate, dense forests, and abundant tick populations, particularly *Ixodes ricinus* and *Dermacentor reticulatus*. These ticks thrive in urban parks, forests, and recreational green spaces, which are commonly visited by dogs and their owners increasing the risk of exposure to tick-borne pathogens. According to the European Pet Food Industry Federation (FEDIAF), 75.8% of Lithuanian households own at least one dog. Data from FEDIAF and various Lithuanian institutions indicate that there could be a total of 550,000–580,000 dogs in Lithuania, a significant portion of which resides in Kaunas, the country's second-largest city. This study investigated the prevalence of tick-borne pathogens, specifically *Babesia canis* and *Anaplasma phagocytophilum*, in dogs attending a veterinary clinic in Kaunas. Blood samples were collected from 203 dogs, including pet and hunting breeds (Lithuanian Hounds), during 2023–2024. Pathogen detection was performed using serological and molecular methods, complemented by morphological blood examinations. Sequence analysis was used to characterize pathogen strains. The results showed that *B. canis* DNA was detected only in pet dogs, with a prevalence of 50.0%. *Anaplasma phagocytophilum* was found in both pet and hunting dogs, with prevalence rates of 27.5% and 4.4%, respectively. Despite higher exposure to tick habitats, hunting dogs had lower pathogen prevalence, likely due to the more rigorous preventive measures taken by their owners to protect against tick bites. This study underscores the importance of routine screening and preventive strategies to reduce the risk of tick-borne diseases in urban canine populations in Lithuania.

Funding: This study was supported by Research Council of Lithuania (Grant No. S-MIP -23/19).

PP 035

Molecular insights into pathogens detected in ectoparasites of companion animals in Sylhet, Bangladesh

T. C. Nath[1], *H. Khanum*[2], *J. U. Bhuiyan*[1]
[1]Sylhet Agricultural University, Parasitology, Sylhet, Bangladesh
[2]Dhaka University, Zoology, Dhaka, Bangladesh

Introduction: Vector-borne pathogens (VBPs) pose significant threats to public health, with ticks and fleas as primary vectors. In Bangladesh, the growing population of free-roaming dogs and cats increases health risks. This study aimed to investigate the diversity of tick and flea species infesting dogs and cats in Sylhet, Bangladesh, and identify VBPs.

Methods: A total of 112 dogs and 48 cats were examined for ectoparasite infestations. Ticks and fleas were collected, identified morphologically, and subjected to DNA extraction. Polymerase chain reaction (PCR) was performed with gene-specific primers to amplify VBPs.

Results: Among dogs, 46 (41.1%) had ticks, 19 (16.9%) had fleas, and 12 (10.7%) were co-infested. In cats, 9 (18.7%) had ticks, 14 (29.2%) had fleas, and 3 (6.2%) were co-infested. Tick species in dogs included *Rhipicephalus sanguineus*, *Ixodes ricinus*, and *Haemaphysalis longicornis*. Flea infestations were primarily caused by *Ctenocephalides canis* and *C. orientalis*. In cats, ticks included *R. sanguineus* and *I. ricinus*, while fleas were predominantly *C. felis*, with fewer instances of *C. orientalis* and *Xenopsylla cheopis*. Pathogen detection in dogs revealed *Babesia gibsoni* as the most prevalent, followed by *Hepatozoon canis*, *Anaplasma platys*, *Ehrlichia canis*, *B. canis*, and *Rickettsia felis*. In cats, *R. felis* was predominant, with single detections of *R. massiliae*, *R. conorii*, and *Bartonella elizabethae*. Sequence analysis showed *B. gibsoni* and *H. canis* from Sylhet were genetically similar to isolates from Assam and Siliguri, India, confirming shared strains across borders.

Conclusion: This study highlights the ectoparasite diversity and VBPs in Sylhet's dogs and cats, underscoring zoonotic risks. Molecular findings reveal cross-border genetic similarities, emphasizing the need for collaborative control efforts. Enhanced surveillance and integrated measures are critical to mitigate vector-borne disease risks in Bangladesh and neighboring regions.

PP 037

Retrospective hematological analysis of *Babesia canis* infections in dogs in Germany (2018–2024)

C. Eisenecker[1], *A. Moritz*[1,2], *I. von Hohnhorst*[2], *C. Strube*[3], *E. Müller*[4], *I. Schäfer*[5]
[1]Justus-Liebig-University Giessen, Clinic of Small Animals-Internal Medicine, Giessen, Germany
[2]Justus-Liebig-University Giessen, Department of Veterinary Clinical Sciences, Clinical Pathology, and Clinical Pathophysiology, Giessen, Germany
[3]University of Veterinary Medicine Hanover, Institute for Parasitology, Centre for Infection Medicine, Hanover, Germany
[4]Laboklin GmbH & Co. KG, Bad Kissingen, Germany
[5]Laboklin GmbH & Co. KG, Department of Clinical Pathology, Bad Kissingen, Germany

Infections with *Babesia* (*B.*) *canis* are of rising clinical and epidemiological importance in Germany, with peaks in spring and autumn in accordance with *Dermacentor reticulatus* activity.
The aim of our retrospective study was to analyze hematological parameters in dogs tested positive for *B. canis* by PCR.

Dogs tested PCR-positive for *B. canis* and negative for *Anaplasma phagocytophilum* in the period from January 2018 to October 2024 were included, if data regarding hematocrit, leukocytes, and platelets were available. Regionality was assessed by the postal code of the veterinarian. $P<0.05$ was considered statistically significant.

Information regarding stays abroad was available in 120/318 dogs (37.8%, no stays abroad n=66/55.0%, import n=40/33.3%, travel n=12/10.0%, import and travel n=2/1.7%). Dogs without stays abroad showed significantly lower leukocytes and thrombocytes, which was also demonstrated non-significantly for hematocrit. Infections were seen throughout the whole year (autumn n=197/61.9%, spring n=67/21.1%, winter n=30/9.4%, summer n=24/7.5%).

Out of 318 dogs, 164 (51.6%) were located in northeastern, 73 in northwestern (23.0%), 69 in southwestern (21.7%), and 12 (3.8%) in southeastern Germany. Pancytopenia was significantly more common in northeastern compared to northwestern and southwestern federal states. Thrombocytopenia was significantly more severe in northeastern compared to northwestern as well as southwestern to northwestern federal states, and in autumn compared to spring and summer. Whether these regional and seasonal effects apply or are biased by subclinically infected imported dogs is currently being evaluated.

Year-around ectoparasite prophylaxis is strongly recommended as acute *B. canis* infections occurred throughout the whole year in all German federal states.

PP 038

Low Serological Reactivity to *Rickettsia* in dogs from the surroundings of an Atlantic rainforest reserve – the influence of large predators?

M. P. J. Szabó[1], *A. Suzin*[1], *P. H. Nunes*[2], *M. F. Moraes*[3], *S. F. Adami*[2], *M. B. Labruna*[4], *A. Vogliotti*[2]
[1]Universidade Federal de Uberlândia, Uberlândia, Brazil
[2]Universidade Federal da Integração Latino-Americana, Foz do Iguaçu, Brazil
[3]Universidade Estadual Paulista, Jaboticabal, Brazil
[4]Universidade de São Paulo, São Paulo, Brazil

The Iguaçu National Park (INP) is a large Atlantic Forest remnant and hosts at least nine tick species and five *Rickettsia* species. In this study, 138 dogs not restricted to their homes were evaluated for ticks: 116 dogs from five municipalities surrounding the INP and 22 dogs from urban fragments within Foz do Iguaçu city. Seroreactivity against *Rickettsia rickettsii*, *R. parkeri*, *R. amblyommatis*, *R. rhipicephali*, and *R. bellii* 39 dogs was evaluated. A total of 169 ticks were collected, with an infestation prevalence of 26% and 36% in the INP surrounding areas and urban fragments, respectively. Four tick species were identified: *Amblyomma ovale* in dogs from both the surrounding areas and urban fragments, with prevalences of 57% and 3%, respectively; *Rhipicephalus sanguineus* sensu lato (24% and 97%); *Amblyomma coelebs* (5% and 0%); and *Rhipicephalus microplus* (3% and 0%). The mean intensity of infestation in dogs from the surrounding areas and urban fragments was 3 and 8, respectively. The highest seroprevalence was 36% against *R. amblyommatis* in dogs from the surrounding areas and 14% against *R. rickettsii*, *R. amblyommatis*, and *R. rhipicephali* in dogs close to urban fragments. The highest observed titer in dogs from the surrounding areas was 1:512 against *R. parkeri*, *R. amblyommatis*, and *R. rhipicephali*, and 1:256 against *R. rickettsii* in dogs close to fragments. The low infestations and the low prevalence and titers of seroreactivity against *Rickettsia* in dogs, are unexpected findings. These results contrast with the high diversity and prevalence of various *Rickettsia* species in free-living tick populations within the INP. Collectively, the observations suggest a reduced exposure of dogs to INP ticks. Among other factors, the abundance of large predators, specifically jaguars, may contribute to reduced incursions and exposure of dogs. This possibility is supported by observations of hunting of several dogs by jaguars in one of the surrounding municipalities.

PP 039

Introduction of exotic ticks (*Amblyomma geoemydae*) in Sweden along with illegal imported turtles (*Geoemyda spengleri*) from Thailand

G. Grandi[1], *A. Omazic*[2], *A. de Jong*[3,1], *L. Chitimia-Dobler*[4,5]
[1]Swedish University of Agricultural Sciences, Department of Animal Biosciences, Uppsala, Sweden
[2]Swedish Veterinary Agency, Department of Chemistry, Environment and Feed Hygiene, Uppsala, Sweden
[3]Swedish Veterinary Agency, Department of Microbiology, Uppsala, Sweden
[4]Ludwig-Maximilians-Universität, Faculty of Veterinary Medicine, Experimental Parasitology, Department of Veterinary Sciences, Munich, Germany
[5]Fraunhofer Institute of Immunology, Infection and Pandemic Research, Munich, Germany

Illegally importation of animals as pets might be a way to introduce exotic ticks, potential vectors of pathogens and can have an impact on wildlife and/or domestic animals and on humans. Therefore, permanent measures of surveillance and control on imported reptiles are emphasized. In February 2024, 31 black-breasted leaf turtles (*Geoemyda spengleri*) were illegally imported into Sweden from Thailand. The turtles were sent by the Swedish Customs Criminal Department West in Gothenburg to a public aquarium and terrarium (Tropicarium, Kolmården, Östergötland county) to be kept in quarantine. At the Tropicarium the turtles were found to be infested by ticks that were collected and stored in ethanol. The finding (including pictures of the ticks) was reported to the Swedish Veterinary Agency (SVA, Uppsala) using the Report Tick Tool (https://rapporterafasting.sva.se/reporttick), a surveillance system developed by the SVA itself. The collected specimens were submitted to the Agency on 26th of February 2024. The three collected ticks were identified based on morphology and genetics as *Amblyomma geoemydae* nymphs. This discovery demonstrates that SVA's reporting tool can function as an early warning system for newly introduced tick species and to promptly identify exotic tick species which can carry viruses, bacteria, and parasites that are not currently present in the country. Continuous data accumulation will help achieve more robust and comprehensive insights, enhancing the reliability and validity of the findings over time.

PP 040

New insights into occurrence of tick-borne pathogens in Zambia

C. Makayi[1], *S. Simainga*[2], *K. Mertens*[3], *L. Chitimia-Dobler*[4,5,6], *G. Dautu*[2], *H. Brangsch*[3]
M. Simuunza[1,7], *M. H. Groschup*[8], *A. Schulz*[8]
[1]University of Zambia, Department of Disease Control, Lusaka, Zambia
[2]Central Veterinary Research Institute, Lusaka, Zambia
[3]Friedrich-Loeffler-Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany
[4]Bundeswehr Institute of Microbiology, Munich, Germany
[5]Fraunhofer Institute of Immunology, Infection and Pandemic Research, Penzberg, Germany
[6]Ludwig-Maximilians-Universität, Department of Veterinary Sciences, Munich, Germany
[7]University of Zambia, Africa Centre of Excellence (Infectious Diseases), Lusaka, Zambia
[8]Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases, Greifswald, Germany

Background: Ticks are important ectoparasites for both humans and animals and can also transmit a wide range of different viral, bacterial and parasitic pathogens, which are commenly known as “tick-borne pathogens” (TBPs). In Zambia (Southern Africa), a number of studies have been conducted on TBPs, but information on their distribution and genetic variation is still incomplete.

Materials and Methods: Between 2022 and 2023, 588 ticks were collected. The ticks were collected from the ground via flagging as well as from different domestic (cattle) and wild host species (buffaloes and tortoises). After tick species identification and DNA/RNA extraction, the samples were tested for viral (orthonairo-, orthoflavi- and arenaviruses) and bacterial (*Rickettsia* (*R.*), *Anaplasma* (*A.*), *Ehrlichia* (*E.*) and *Coxiella* (*C.*)) pathogens using different PCR assays. Subsequently, positive samples were sequenced and analyzed based on different genes (16S; 23S; *ompB*; *gltA*; *sca4*).

Results: Apart from some *Hyalomma* and *Amblyomma* species, most of the ticks collected belonged to the genus *Rhipicephalus,* in which *Rhipicephalus* (*Rh.*) *appendiculatus* made up the largest proportions. No viruses were detected inside the ticks, but *Rickettsia* (*R. africae*, *R. aeschlimannii*, *R. sibirica, R. tamurae*/*R. monacensis*), *Anaplasma* (*A. phagocytophilum*, *A. bovis*, *A. marginale*, *A. platys*) and *Ehrlichia* species (*E. ruminantium*, *E. chaffeensis*) were most frequently found.

Conclusions: Many of the pathogens found had already been described in Zambia. Nevertheless, there were some unexpected findings, such as *R. sibirica* (based on *ompB*; 23S) found in ticks from cattle or *E. chaffeensis* (based on 16S) that was present in a questing *Rh. appendiculatus* tick. These results indicate that some pathogens might be more widespread than previously assumed, contrary to earlier assumptions. Alongside these rarely described pathogens in Africa, further studies should be also carried out on the detection of TBPs of viral origin.

PP 041

Investigation of a piroplasm outbreak in a herd of horses in Southern Germany

R. Vogt[1], *K. Fachet-Lehmann*[1], *A. Lindau*[1], *M. Grünke*[1], *S. Müller*[2], *U. Mackenstedt*[1]
[1]University of Hohenheim, Department of Parasitology, Stuttgart, Germany
[2]Tierseuchenkasse Baden-Württemberg, Pferdegesundheitsdienst, Stuttgart, Germany

Equine piroplasmosis is a tick-borne disease caused by the protozoan parasites *Babesia caballi* and *Theileria equi*, transmitted by hard ticks (Ixodidae) that causes symptoms such as fever, anaemia, jaundice, and, in severe cases, can be fatal. In October 2022, 18 out of 34 horses in one herd in the district of Rastatt, Baden-Wuerttemberg, were infected with at least one of the two pathogens and three horses had to be euthanized due to severe symptoms. The aim of this case study was to determine the pathogen prevalence in the herd of horses and in the local tick population of *Dermacentor reticulatus*. Therefore, blood samples were collected in September 2023 and in March 2024 for molecular and serological tests and blood smears were made to screen microscopically for both pathogens. Molecular testing of the blood samples showed infection rates of 53%/26% for *B. caballi* and of 71%/65% for *T. equi* (2023/2024). cELISA revealed infection rates of 74%/88% for *B. caballi* and 62%/65% for *T. equi*. *Babesia caballi* and *T. equi* stages could both be identified in the blood smears. Moreover, horses could be tested positive by PCR and cELISA in March 2024, which had been tested negative in September 2023 for both pathogens. Additionally, ticks were collected from the horses' pastures from November 2022 until March 2024 periodically and examined for piroplasms via PCR. In total over 1,000 ticks were collected and 965 adult *D. reticulatus* were examined. Eight ticks were tested positive for *B. caballi*, whereas *T. equi* was not detected in any tick. However, the positive tested ticks came from pastures on which the horses grazed during and directly before the outbreak in 2022. The origin of equine piroplasmosis could not be finally clarified, but an imported horse from Spain is suspected to have introduced both pathogens into the herd. These results highlight the increasing importance of piroplasms, which can become established in Germany due to the widespread distribution of *D. reticulatus*.

PP 042

Molecular detection *Theileria annulata* in cattle from South Kazakhstan

K. Mukanov[1], *D. Kamalova*[1], *A. Ostrovskii*[1], *E. Shevtsova*[1]
[1]National Center for Biotechnology, Laboratory of Applied Genetics, Astana, Kazakhstan

Introduction: *Theileria annulata* is a tick-borne apicomplexan parasite that affects cattle. It is endemic in numerous tropical and subtropical regions, including Kazakhstan. The objective of this study is to detect *T. annulata* infection in bovine blood samples from southern Kazakhstan using conventional polymerase chain reaction (PCR).

Methodology: This study presents the results of PCR testing for *T. annulata*. A total of 2,491 whole blood samples were collected from cattle older than three years of age across five southern regions of Kazakhstan. A standard PCR assay targeting the *T. annulata enolase* gene was conducted to screen the samples.

Results: The study revealed an overall prevalence of *T. annulata* infection in the sampled cattle population of 45%. The region-specific results demonstrated a prevalence of 81% in the Turkestan region (n=598), 56% in the Zhambyl region (n=347), and 24.6% in the Kyzylorda region (n=170). The Almaty region exhibited the lowest prevalence, with a rate of 5% (13 positive samples out of 245). No positive cases were identified among the 200 samples tested in the Zhetysu region.

Conclusion: The highest prevalence of *T. annulata* was observed in the Turkestan, Zhambyl, and Kyzylorda regions, likely due to favorable climatic conditions that facilitate the proliferation of vectors such as ixodid ticks. These findings highlight that the geographical distribution of *T. annulata* is shaped by various factors, including climatic conditions, vector population density, and the effectiveness of preventive measures implemented in each region.

PP 043

Distribution of *Babesia* spp. in cattle across the Southern and Central regions of Kazakhstan

N. Tursunbay[1], A. Akhmetova[1], A. Dauletov[1], B. Abdigulov[1], A. Ryskeldina[1], A. Ostrovskii[1]
M. Kadyrova[1], A. Kairzhanova[1]
[1]National Center for Biotechnology, Applied Genetic Laboratory, Astana, Kazakhstan

In the southern regions of Kazakhstan, cattle babesiosis is an endemic disease that causes significant economic losses to agriculture. However, data on the distribution and species composition of *Babesia spp*. remain sporadic, unsystematic, and potentially outdated. Considering the landscape, climate features, and vector composition, cases of babesiosis are predominantly recorded in the southern regions of the country.

This study presents the results of an assessment of the species diversity of *Babesia spp*. conducted through sequencing in cattle from the southern (Turkestan and Zhambyl regions), central (Karaganda and Ulytau regions), and northern (North Kazakhstan region) parts of Kazakhstan. A total of 1,238 blood samples were collected from cattle older than three years in each region. DNA was extracted using the silica-based method, and primers targeting the *18S rRNA* gene were employed to detect *Babesia spp*.

The findings revealed significant regional differences in the prevalence of infection. Positive samples were identified in the southern regions (Turkestan and Zhambyl regions) with an infection rate of 3.8% and in the central part of Kazakhstan (Ulytau region) with an infection rate of 3.3%. In contrast, all samples from the northern (North Kazakhstan region) and central (Karaganda region) regions were negative. Species identification conducted using Sanger sequencing revealed that *Babesia bigemina*, *Babesia major*, and *Babesia occultans* were the most prevalent species. The detection of positive samples in the Ulytau region, which borders high-risk regions (Turkestan and Zhambyl regions), suggests a potential risk of further spread of *Babesia spp*. to the central regions of Kazakhstan.

This study underscores the importance of molecular genetics methods in monitoring for accurate species identification, updating the epidemiological situation of piroplasmosis in cattle, and improving control measures.

PP 044

Ticks on cats and dogs in Northern Cyprus

A. Seyer Çağatan[1], A. Keskin[2], K. Mumcuoglu[3], A. Taylan Ozkan[5, 6]
[1]Cyprus International University, Medical and Clinical Microbiology, Nicosia, Cyprus
[2]Tokat Gaziosmanpaşa University, Biology, Faculty of Science & Art, Tokat, Turkey
[3]The Hebrew University-Hadassah Medical School, Microbiology and Molecular Genetics, Jerusalem, Israel
[4]TOBB University of Economics and Technology, Medical Microbiology, Ankara, Turkey
[5]Faculty of Medicine, TOBB University of Economics and Technology
[6]Faculty of Medicine, Cyprus International University

Background: The tick fauna of Northern Cyprus is poorly known. In this study, we aimed to investigate the ticks of peridomestic cats and dogs in this part of the island.

Methods: The study was conducted between the years 2021-2023 and ticks were collected from 14 cats and 118 dogs in the areas of Lefkosia, Girne and Güzelyurt. Each animal was examined in the head and neck area, isolated ticks were preserved in 70% ethyl alcohol.

Results: A total of 305 ticks were collected: 34 from cats and 271 from dogs. The most common tick on cats was *Rhipicephalus turanicus* (n=18, 53%), followed by *Ixodes ricinus* (n=7, 20.6%) and *Rhipicephalus sanguineus* (n=1, 2.8%). In addition, eight nymphs (23.6%) of *Rhipicephalus* spp. were collected from three cats. Out of the 271 ticks collected from dogs 210 were *R. turanicus* (77.49%), 54 *R. sanguineus* (19.93%), and seven *I. ricinus* (2.58%). Three of the *R. sanguineus* ticks collected from a dog were of the *rutilus* type.

Conclusions: This study provides an understanding of the prevalence of ticks infesting cats and dogs in Northern Cyprus. *Ixodes ricinus* was reported for the first time in Cyprus while *R. turanicus* seems to be the most common tick in cats and dogs in the examined regions.

PP 045

Is anaplasmosis endemic in smallholder cattle and buffaloes in Pakistan – evidence from molecular phylogenetic analysis

A. Ghafar[1], *A. Jabbar*[1]
[1]University of Melbourne, Melbourne Veterinary School, Werribee, Australia

Bovine anaplasmosis, caused by *Anaplasma marginale*, is a major tick-borne disease associated with significant production losses, anaemia, abortion, and mortality in adult animals, particularly in smallholder farming systems in the tropics and subtropics. In addition, prolonged convalescence periods and subclinical carrier infections are also common among cattle that survive the clinical disease. In this study, we aimed to detect and characterise *A. marginale* in the bovine population in Pakistan by targeting the major surface protein B gene (*msp1β*) using a nested PCR approach. A total of 323 blood samples was collected from apparently healthy cattle ($n = 176$) and buffaloes ($n = 147$) across six districts in Pakistan and analysed using molecular phylogenetics. Of the 323 DNA samples tested, 136 (42.1%) were positive for *A. marginale* on the agarose gel. The prevalence was significantly higher in cattle (64.2%; 113/176) than in buffaloes (15.6%; 23/147). Similarly, females (43.6%; 109/250) were more frequently infected than males (37%; 27/73). Pairwise nucleotide sequence differences of 0.6%–11.6% were observed among the *msp1β* sequences (216 bp). NCBI BLAST results and phylogenetic analyses revealed that *msp1β* sequences from Pakistan clustered with reported sequences from Brazil, Thailand, South Africa and the USA. This study represents the first comprehensive investigation of bovine anaplasmosis across diverse agroecological zones of Pakistan, offering valuable insights into the prevalence of this important infection in smallholder farming systems. It also highlights the potential presence of endemic stability against *A. marginale* in the cattle population of this country. Future studies to characterise and quantify *A. marginale* through qPCR will provide a deeper understanding of infection dynamics. Furthermore, it is crucial to assess the economic impact of subclinical carrier infections within the bovine population on the livelihoods of resource-poor communities.

PP 046

Presence of *Anaplasma phagocytophilum* in lambs at post mortem is associated with an increased likelihood of co-infection with Pasteurellaceae bacteria and the diagnosis of pneumonia

J. Gilmore[1], *K. O Keefe*[2], *C. Hurley*[2], *M. Hennessy*[2], *A. N. Lucena*[2], *M. Casey*[3], *C. Mc Dermott*[3]
K. Beirne[3], *S. Fagan*[2], *J. Mee*[4], *T. Keady*[5], *S. Mc Gettrick*[2], *A. M. Flaherty*[2], *R. Froehlich*[2]
M. Sheehan[2], *A. Zintl*[3]
[1]FarmLab Diagnostics, Elphin, Ireland
[2]Department of Agriculture Food and the Marine, Dublin, Ireland
[3]University College Dublin, Veterinary Medicine, Dublin, Ireland
[4]Teagasc, Cork, Ireland
[5]Teagasc, Athenry, Ireland

Introduction

Anaplasma phagocytophilum is the cause of tick-borne fever (TBF) in ruminants. It is associated with general malaise, lack of production, fever, respiratory signs and immunosuppression leading to exacerbated co-infections with other pathogens. It is also a cause of infectious abortion in sheep. Little is known about the prevalence and significance of *A. phagocytophilum* in Irish sheep. This study aimed to assess the prevalence of *A. phagocytophilum* in lamb carcases submitted to 3 Irish Regional Veterinary Laboratories in 2021 and 2022 using qPCR and to determine which co-morbidities it was most strongly associated with.

Materials and methods

A sample from the spleen of every lamb submitted to three Regional Veterinary Laboratories (Sligo, Athlone and Kilkenny) in 2021 and 2022 was analysed by qPCR. Postmortem data, including flock location, flock history, age and diagnosis were recorded for each animal. Chi-square Analysis was used to identify relationships between *A. phagocytophilum* PCR status and other concomitant findings.

Results

A total of 864 samples were analysed, of which 57 showed a TBF-positive result when tested with PCR. Pneumonia was identified as the primary cause of death in 22.8% of TBF-positive and in 13.9% in TBF-negative lambs. Pneumonia causing bacteria from the family *Pasteurellaceae* were identified in 33.3% of TBF positive samples and 17.7% of TBF negative samples. Clostridial disease was diagnosed in 19.3% of TBF positive and 13.9% of TBF negative cases.

Conclusions

A diagnosis of pneumonia and the identification of pneumonia-causing pathogens at post mortem appears to be associated with co-infection with *A. phagocytophilum*. The study also suggests that infection with *A. phagocytophilum* may increase the risk of death due to clostridial disease, whereas lambs which test negative for *A. phagocytophilum* have an increased risk of presenting for post mortem with gastrointestinal related disorders.

OP 031

Tick-borne pathogens in Poland from a clinical perspective

A. Moniuszko[1]
[1]Medical University of Białystok, Department of Infectious Diseases and Neuroinfections, Bialystok, Poland

In Poland, tick-borne pathogens pose significant public health concerns, primarily due to the increasing incidence of tick-borne diseases. There has been a notable increase in reported cases of tick-borne diseases in Poland, likely influenced by socio-economic and environmental changes, such as climate variations that affect tick populations and their distribution.

Lyme Disease incidence is high (25 244 cases in 2023, incidence: 66.96 per 100 000 inhabitants) and still there are many dilemma regarding over- and underdiagnosis of the disease. There are many patients who search for non-standardized treatment with ILADS method, either in Poland or abroad.

Tick-borne encephalitis number of cases is also increasing: in 2023 it was 659 (1.75 per 100 000 inhabitants) in comparison to 265 (0.69 per 100 000 inhabitants) in 2019. The disease is being diagnosed in new voivodships, where it was not observed before. Even if the vaccination is available for many years and there is an increase in vaccination dosage given to the patients observed (in 2019 – 87 917 and in 2023 – 102 147 doses), still vaccination rate is low and people suffer from short and long-lasting sequelae after TBE. In the Department of Infectious Diseases and Neuroinfections, Medical University of Bialystok, Poland over last 30 years 1 654 patients were hospitalized.

Anaplasmosis, rickettsioses and tularemia are also more and more frequently diagnosed in Poland, mostly due to the development of new diagnostics methods and rising awareness of physicians and society about these diseases.

In summary, tick-borne pathogens represent a significant clinical challenge in Poland, requiring comprehensive public health strategies, effective diagnosis, and management to mitigate their impact on single patient and public health.

OP 032

Impact of acute Lyme disease in children

L. Nigrovic[1], *M. Ladell*[2], *L. Chapman*[3], *F. Balamuth*[4], *D. Neville*[5], *A. Kharbanda*[6], *S. Hadley*[1]
A. Thompson[7]
[1]Boston Children's Hospital, Pediatrics, Boston, MA, United States
[2]Children's Hospital of Wisconsin, Milwaukee, WI, United States
[3]Rhode Island Hospital, Providence, RI, United States
[4]Children's Hospital of Philadelphia, Philadelphia, PA, United States
[5]Children's Hospital of Pittsburgh, Pittsburgh, PA, United States
[6]Children's Minnesota, Minneapolis, MN, United States
[7]Nemours Children's Hospital, Wilmington, DE, United States

Background: While some children with Lyme disease have only mild symptoms, others require invasive procedures, parenteral antibiotics or hospitalization.
Objective: Our goal was to describe the clinical impact of acute Lyme disease in a geographically diverse pediatric cohort.

Methods: We performed a prospective cohort study of children 1 to 21 years of age who presented to one of eight Pedi Lyme Net emergency departments between 2015 and 2024 for suspected Lyme disease. For this substudy, we selected those with confirmed Lyme disease, defined by either a clinician diagnosed erythema migrans (EM) lesion measuring at least 5 cm in diameter or positive two-tier Lyme disease serology within 30 days of enrollment. We defined Lyme disease stage as follows: early-localized (single EM lesion), early-disseminated (multiple EM lesions, facial palsy, meningitis, carditis) or late (arthritis).

Results: Of 5,534 children enrolled, 1,346 (24.3%) had confirmed Lyme disease. Of these, 109 (8.1%) had early-localized, 536 (39.8%) early-disseminated disease and 701 (52.1%) late Lyme disease. The median age was 8 years [interquartile range (IQR) 5-12 years] and 846 (62.9%) were male. Of the 302 (22.4%) who were hospitalized for Lyme disease management, the median duration of hospital stay was 2 days (IQR 1-3 days). Of those with early-disseminated Lyme disease, 82 (15.3%) had a lumbar puncture performed and, of those with late Lyme disease, 158 (22.5%) had an invasive joint procedure (arthrocentesis and/or joint wash-out). Overall, 139 (10.3%) of children with Lyme disease received at least one dose of parenteral antibiotics.

Conclusions: Acute Lyme disease has substantial impact for many children, resulting to invasive procedures, parenteral antibiotic and/or hospitalization. Strategies to prevent Lyme disease have the potential to substantially reduce the impact on children.

OP 033

KillaRay – a novel approach preventing *Borrelia afzelii* amplification in human skin model

D. Wężyk[1], S. Waszkiel[1], P. Wachulak[2], T. Chmielewski[3], B. Grala[4], J. Kania[4], B. Mierzejewski[5]
A. Bajer[1]
[1]University of Warsaw, Eco-Epidemiology of Parasitic Diseases, Warsaw, Poland
[2]Military University of Technology, Institute of Optoelectronics, Warsaw, Poland
[3]National Institute of Public Health, Department of Parasitology and Vector-Borne Diseases, Warsaw, Poland
[4]Military Institute of Medicine-National Research Institute, Department of Pathology, Warsaw, Poland
[5]University of Warsaw, Department of Cytology, Warsaw, Poland

Ticks are vectors for numerous human diseases, with Lyme borreliosis being the most common tick-borne disease in Europe, affecting an estimated 650,000 to 850,000 people annually. During feeding, ticks attach to the skin for several days, creating micro-incisions in the skin and blood capillaries and depositing pathogens into a confined blood pool within the skin. Currently, no methods exist for disinfecting the site of a tick bite to prevent infection.

This study evaluated the effectiveness of photoinactivation in preventing *Borrelia afzelii* infection using an in vitro human skin model. Human skin biopsies were intradermally inoculated with *B. afzelii* bacteria at two concentrations (10^6, 10^7), followed by a collimated beam of light exposure after 30 mins and/or 12 hours post-inoculation. Bioptates were maintained for seven days at temp. 34°C and 5% CO2. For the identification of bacteria presence/absence in skin bioptates, bacteria cultures were set in BSH medium from both the biopsis media and the skin fragments and monitored over an eight-week period to detect any viable spirochetes. Positive controls (skin bioptates inoculated with *B. afzelii* and not exposed to collimated beams of light) were also set. For the detection of bacteria DNA, PCR were conducted on samples from both the media and the skin samples at the end of the experiment.

Results: collimated beam of light effectively eradicated viable spirochetes in treated samples, with no positive bacteria cultures in exposed groups. In untreated controls, *B. afzelii* presence was confirmed through both cultures and PCR. These findings suggest that photoinactivation may serve as a promising, non-invasive method to disinfect tick bite sites and prevent spirochaete infection.
Acknowledgements: The study was funded by the University of Warsaw IDUB New Ideas 2B, grant no. IDUB-622-317/2022 (AB).

OP 034

Duplex PCR assay to determine sex and mating status of *Ixodes scapularis*, vector of the Lyme disease pathogen

I. Ronai[1,2], *J. Frederick*[3,4], *A. Thompson*[5,4], *P. Sharma*[3], *M. Yabsley*[5,6,4], *U. Pal*[7], *T. Glenn*[3,8,4]
C. Extavour[1,2,9], *K. Chong*[1,10]
[1]Harvard University, Department of Organismic and Evolutionary Biology, Cambridge, MA, United States
[2]Howard Hughes Medical Institute, Chevy Chase, MD, United States
[3]University of Georgia, Department of Environmental Health Science, Athens, GA, United States
[4]University of Georgia, Odum School of Ecology, Athens, GA, United States
[5]University of Georgia, Department of Population Health, Athens, GA, United States
[6]University of Georgia, Warnell School of Forestry and Natural Resources, Athens, GA, United States
[7]University of Maryland, Department of Veterinary Medicine, College Park, MD, United States
[8]University of Georgia, Institute of Bioinformatics, Athens, GA, United States
[9]Harvard University, Department of Molecular and Cellular Biology, Cambridge, MA, United States
[10] Harvard University, Museum of Comparative Zoology, Cambridge, MA, United States

Ticks are a major health threat to humans and other animals, through direct damage, toxicoses, and transmission of pathogens. An estimated half a million people are treated annually in the United States of America for Lyme disease, a disease caused by the bite of a black-legged tick (*Ixodes scapularis*) infected with the bacterial pathogen *Borrelia burgdorferi*. This tick species also transmits another six human-disease causing pathogens, for which vaccines are currently unavailable. While *I. scapularis* are sexually dimorphic at the adult life stage, the DNA sequence differences between male and female *I. scapularis* that could be used as a sex-specific marker have not yet been established. We determine the sex-specific DNA sequences for *I. scapularis* (male heterogametic system with XY), using whole-genome resequencing and restriction site-associated DNA sequencing. Then we identify a male-specific marker that we use as the foundation of a molecular sex identification method (duplex PCR) to differentiate the sex of an *I. scapularis* tick. In addition, we provide evidence that this molecular sexing method can establish the mating status of adult females that have been mated and inseminated with male-determining sperm. Our molecular tool allows the characterization of mating and sex-specific biology across development for *I. scapularis*, a major pathogen vector, which is crucial for a better understanding of their biology and controlling tick populations.

OP 035

From phylogenomics to functional evolution – insights into the adaptation of ticks to a blood-feeding environment using comparative sialomics

B. J. Mans[1], *D. de Klerk*[1], *R. Pienaar*[1]
[1]Agricultural Research Council - Onderstepoort Veterinary Research, Epidemiology, Parasites and Vectors, Onderstepoort, South Africa

A main aim in systematics is the construction of accurate systematic frameworks that represent true phylogenies or evolutionary relationships within lineages. This allows for identification of character origins in the last common ancestor, loss or gain of characters within lineages and inference on what the appearance of characters mean for evolution of biological traits such as blood feeding, or emergence of biological function. For ticks a concerted effort using mitochondrial genomics and phylogenomics is underway, with significant progress that allow construction of a systematic framework with a high degree of confidence. Evidence support recognition of three tick families with a basal relationship for the Nuttalliellidae to the Argasidae and Ixodidae. Within the Argasidae, a division between genera in the Argasinae and Ornithodorinae is evident with surprising groupings of genera. Hard ticks continue to show the emergence of new genera that confounds traditional classifications of historical lineages such as the Amblyomminae, Haemaphysalinae and Rhipicephalinae. This emphasizes the importance of an accurate systematic framework to reliably infer the origins of protein function. The characterization of sialomes from diverse genera gives insight into the diversity of protein families and the presence of unique or shared orthologs in different lineages. Salivary gland sialomes have been sequenced for the major argasid and ixodid genera as well as *Nuttalliella*. This includes for the Argasinae: *Alveonasus*, *Argas*, *Ogadenus*, *Proknekalia*, *Secretargas* and the Ornithodorinae: *Alectorobius*, *Ornithodoros* and *Pavlovskyella*. For ixodids, transcriptomes have been sequenced for *Amblyomma*, *Dermacentor, Hyalomma*, *Ixodes* and *Rhipicephalus*. Comparative transcriptomics indicate conservation of main families, but few conserved functional orthologs for secretory proteins. This supports the evolution of different adaptive strategies after divergence of the main tick families.

OP 036

Redescription of *Alloceraea cretacea* with an additional nymphal fossil added to this species

L. Chitimia-Dobler[1,2], *C. Mey*[3], *D. Harms*[4], *J. Hammel*[5], *J. A. Dunlop*[6], *U. Kotthoff*[4], *B. J. Mans*[7,8,9]
[1]Fraunhofer Institute of Immunology, Infection and Pandemic Research, Munich, Germany
[2]Ludwig-Maximilians-Universität, Experimental Parasitology, Department of Veterinary Sciences, Faculty of Veterinary Medicine, Munich, Germany
[3]Universität Hamburg, Institute for Geology, Hamburg, Germany
[4]Leibniz-Institute for the Analysis of Biodiversity Change (LIB), Hamburg, Germany
[5]Helmholtz-Zentrum Hereon, Institute of Materials Physics, Geesthacht, Germany
[6]Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany
[7]Agricultural Research Council-Onderstepoort Veterinary Research, Epidemiology, Parasites and Vectors, Onderstepoort, South Africa
[8]University of South Africa, Department of Life and Consumer Sciences, Pretoria, South Africa
[9]University of the Free State, Department of Zoology and Entomology, Bloemfontein, South Africa

A fossil assigned to the extant hard tick genus *Haemaphysalis* CL Koch (1844) (Ixodida: Ixodidae) was described from the Late Cretaceous (ca. 99 Ma) Burmese amber of Myanmar. *Haemaphysalis* (*Alloceraea*) *cretacea* is considered the oldest and only fossil representative of this genus. Recently, the subgenus *Alloceraea* was raised to genus level, that would place the fossil in this new genus. However, significant criticism was raised regarding misidentification with controversial discussions by experts in the field of tick morphology. Microtomography of the existing and new specimens allowed new perspectives on these controversies. The obtained images and videos were investigated and compared with extant related species. A new nymph with similar morphology to *Al. cretacea* is described. Both specimens are considered *Alloceraea* nymphs based on a series of morphological aspects: no genital aperture, eyeless, 11 festoons, coxa I simple and with a short, widely triangular spur, and specific morphological feature for the structurally primitive *Alloceraea*: palpi elongate, with long setae on palps, the hypostome longer than chelicera seen dorsally and the corona visible distally and with a specific distribution of the denticles. While the fossils belong to the *Alloceraea*, fossils of their sister-group, the *Archaeocroton* was also described from Burmese amber. These two genera share a common ancestor with *Haemaphysalis* sensu stricto, indicating a minimum divergence time of at least 100 MYA between these lineages.

OP 037

Similarities between *Ixodes ricinus* and *Ixodes inopinatus* genomes and horizontal gene transfer from their endosymbionts

O. Jlassi[1,2]
[1]Pasteur Institute of Tunisia/University of Sciences of Bizerte; Tunisia, Biology, Tunis, Tunisia
[2]National Institute of Public Health and Environment, Dier and Vector (D&V), Utrecht, Netherlands

The taxa *Ixodes ricinus* and *Ixodes inopinatus* are sympatric in Tunisia. The genetics underlying their morphological differences are unresolved. In this study, ticks collected in Jouza-Amdoun, Tunisia, were morphologically identified and sequenced using Oxford Nanopore Technologies. Three complete genome assemblies of *I. inopinatus* and three of *I. ricinus* with BUSCO scores of ~98% were generated, including the reconstruction of mitochondrial genomes and separation of both alleles of the *TRPA1*, *TROSPA* and *calreticulin* genes. Deep sequencing allowed the first descriptions of complete bacterial genomes for "*Candidatus* Midichloria mitochondrii", *Rickettsia helvetica* and *R. monacensis* from North Africa, and the discovery of extensive integration of parts of the *Spiroplasma ixodetis* and "*Ca*. M. mitochondrii" into the nuclear genome of these ticks. Phylogenetic analyses of the mitochondrial genome, the nuclear genes, and symbionts showed differentiation between Tunisian and Dutch ticks, but high genetic similarities between Tunisian *I. ricinus* and *I. inopinatus*. Subtraction of the genome assemblies identified the presence of some unique sequences, which could not be confirmed when screening a larger batch of *I. ricinus* and *I. inopinatus* ticks using PCR. Our findings yield compelling evidence that *I. inopinatus* is genetically highly similar, if not identical, to sympatric *I. ricinus.* Defined morphological differences might be caused by extrinsic factors such as micro-climatic conditions or bloodmeal composition. Our findings support the existence of different lineages of *I. ricinus* as well of its symbionts/pathogens from geographically dispersed locations.

OP 038

Octopamine and adrenergic-like octopamine receptors in *Ixodes ricinus* salivary glands

S. Rakotobe[1]*, O. Hajdusek*[2]*, J. Perner*[2]*, L. Simo*[1]
[1]ANSES – INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, France
[2]Czech Academy of Sciences, Biology Centre, Institute of Parasitology, Ceske Budejovice, Czech Republic

The neurotransmitter octopamine, a structural and functional analogue of the vertebrate adrenaline, plays essential roles in various aspects of invertebrate physiology. Here, using in silico approaches we identified that in tick genomes two types of adrenergic-like octopamine and two types of tyramine receptors are present. Among those, transcripts of *Ixodes ricinus* α-2 adrenergic-like octopamine receptor (Octα2R) and its β counterpart (OctβR) have been chemically synthetized and functionally tested in the heterologous expression system. For both receptors, octopamine was the most potent activator 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. Subsequent immunostaining with an antibody against the tyramine β-hydroxylase (TBH), an enzyme synthetizing octopamine, confirmed the reaction in three pairs of those neurons. In salivary glands octopamine-like immunoreaction has been detected on luminal site of type II and III acini as scattered patches in close association with a single myoepithelial cell. In addition, double staining of octopamine with known markers for *Ixodes* salivary gland axons, the neuropeptides and invertebrate-like dopamine receptor, showed distinct staining patterns. Interestingly the anti-TBH antibody recognized robust basal axons reaching both type II and III acini as well as fine scattered patches in close association with octopamine-like immunosignal in the same structures. Thus our results indicate axonal delivery of octopamine to *Ixodes* salivary gland. Finally, injection of octopamine to partially fed *Ixodes* females triggered saliva secretion, however with about 10 minutes delay after the injection. Our pioneer study represents a steppingstone for a deeper molecular and biochemical exploration of the octopamine physiology in tick salivary glands.

OP 039

A sTicky Situation – on the phase separation and ageing of disordered proteins from tick cement cone

K. Ganar[1], M. Nandy[1], P. Turbina[1], I. Dijkgraaf[2], S. Deshpande[1]
[1]Wageningen University, Laboratory of Physical Chemistry and Soft Matter, Wageningen, Netherlands
[2]Maastricht University, Department of Biochemistry, Maastricht, Netherlands

Hard ticks feed on their host for multiple days. To ensure firm attachment, they secrete a protein-rich saliva that eventually forms a solid cement cone. The underlying mechanism of this liquid-to-solid transition is currently not understood. In this talk, I will focus on the phase transitions of a disordered glycine-rich protein (GRP) from a protein family that is prominent in the cement cone. I will show that GRP undergoes liquid-liquid phase separation via simple coacervation to form biomolecular condensates in salty environments. Cation-pi and pi-pi interactions mediated by periodically placed arginine and aromatic amino acid residues are the primary driving forces that promote phase separation. Interestingly, GRP condensates exhibit ageing by undergoing liquid-to-gel transition over time and exhibit adhesive properties, thus resembling the naturally occruing cement cone. Our findings provide a starting point to gain insights into the bioadhesion of ticks, develop novel tick control strategies, and towards biomedical applications such as tissue sealants.
Reference: Ganar, K.A., Nandy, M., Turbina, P. *et al.* Phase separation and ageing of glycine-rich protein from tick adhesive. *Nat. Chem.* (2024). https://doi.org/10.1038/s41557-024-01686-8

OP 042

Are there 16 species of brown dog ticks? Phylogenies from 59 entire mitochondrial genomes and 157 *cox1* sequences reveal 16 species-level clades in the *Rhipicephalus (Rhipicephalus) sanguineus* group

S. Kelava[1]
[1]Hokkaido University, Department of Parasitology, Sapporo, Japan

The *Rhipicephalus sanguineus* group, known as brown dog ticks, is a globally distributed and highly significant group of ectoparasites, both economically and clinically, particularly for domestic dogs. Despite their importance, the group's taxonomy has been plagued by confusion and inconsistencies for over four decades, largely due to overlapping morphological characteristics and limited genetic resolution in earlier studies.

To address these challenges, we generated 13 new mitochondrial genomes and ITS2 sequences from *R. sanguineus* group ticks collected in Australia, Israel, and Japan. Using phylogenetic analyses of mitochondrial protein-coding genes, rRNA genes, and partial *cox1* sequences, we identified 16 distinct clades (A–P) that we hypothesize represent separate species.

We applied species delimitation protocols, including Automatic Barcode Gap Discovery, Assemble Species by Automatic Partitioning, and Poisson Tree Process, which confirmed the species-level divergence between these clades. Intra-clade genetic differences were consistently below 3.8% (mitogenomes) and 5.1% (*cox1*), while inter-clade differences exceeded 7.7% and 5.1%. Several historical species names, such as *R. linnaei* (clade A) and *R. sanguineus* (clade J), were reassigned, while six clades (F, H, K, M, N, and O) could not be named and may represent novel species.

A haplotype network of partial mitochondrial genes (*cox1*, *cytb*, and *nad2*) revealed significant genetic similarity among geographically distant populations of *R. linnaei*, suggesting recent global dispersal, likely originating in Africa or the Middle East. This hypothesis is supported by the higher genetic diversity observed in African populations compared to those from other regions.

These findings provide much-needed clarity to the taxonomy of the *R. sanguineus* group and offer insights into their evolutionary history and global dispersal patterns.

OP 043

Laboratory crosses of *Rhipicephalus sanguineus* s.s. and *Rhipicephalus hibericus* demonstrate lack of fertile progeny and support species separation

A. Estrada-Peña[1]
[1]Ministry of Human Health, Madrid, Spain

The *Rhipicephalus sanguineus* tick group is a complex of species not yet well resolved in the Palearctic. Some species have been redescribed in the last years (e.g. *Rhipicephalus sanguineus* s.s., *Rhipicephalus pusillus, Rhipicephalus secundus*) or recognised and described as separate entities (e.g. *Rhipicephalus hibericus*). In this study, we present the results of laboratory crosses of *R. sanguineus* s.s. and *R. hibericus*, demonstrating the existence of a hybrid F1 progeny.

Laboratory protocols were carried out in the years 2016 to 2018, as part of the studies leading to the redescription of *R. sanguineus* s.s. (year 2018) and the description of *R. hibericus* (year 2024). Adult *R. sanguineus* s.s. were crossed with specimens later described as *R. hibericus*. Ten males and ten females of each taxon were fed on Giant New Zealand Albino rabbits in capsules placed on the back of hosts. Feeding and detachment were recorded daily. Engorged stages were kept at 25ºC, 80% relative humidity, and 12/12 hours light/darkness.

Adult crosses produced viable F1 progeny. Larvae of F1 hatched in 98% (+/- 1.3) of egg clutches and survived to feed and molt to nymphs. The preoviposition (8 days +/- 1.8), incubation (22 +/- 2.3)), larva-nymph molt (17 +/- 3.1)) and nymph-adult molt (18 +/- 1.9) were statistically similar to those of *R. sanguineus* s.s. (control, $p=0.431$). Measurements of critical morphological features in both taxa and the resulting progeny were statistically similar but for the larval length and width of idiosome; hybrid larval ticks were about 10% smaller than *R. sanguineus* s.s. Males and females of F1 fed and mate but produced non-viable eggs. Clutch of eggs turned dark and dry. No larvae of F2 were obtained, therefore supporting that the previous F1 was a hybrid progeny.

The data on hybridization and the lack of a fertile progeny in the crosses of *R. sanguineus* s.s. and *R. hibericus* are indicative of two different species and support the description of the latter.

OP 044

Rhipicephalus sanguineus sensu stricto (Latreille, 1806) and *Rhipicephalus linnaei* (Andouin, 1826) – Are they also different in susceptibility to acaricides?

G. Klafke[1], B. Rauta de Avelar[2], B. Guimaraes Gava[2], Y. Henrique da Silva[2], P. Teixeira Ferreira[1] J. Reck[1], F. Jongejan[3,4], T. Ribeiro Correia[2], F. Barbour Scott[2]
[1]Instituto de Pesquisas Veterinárias Desidério Finamor, Eldorado do Sul - RS, Brazil
[2]Universidade Federal Rural do Rio de Janeiro, Seropedica, Brazil
[3]TBD International BV, Utrecht, Netherlands
[4]University of Pretoria, Onderstepoort, South Africa

In Brazil, the brown dog ticks *Rhipicephalus sanguineus* sensu stricto and *Rhipicephalus linnaei* have distinct geographical distributions (temperate vs. tropical regions) and roles in tick-borne disease epidemiology. Despite these distinctions, their control relies on synthetic acaricides such as permethrin, fipronil, and isoxazolines. Resistance to pyrethroids and phenylpyrazoles has been reported in both species. This study evaluated interspecific susceptibility differences to establish baselines for acaricide resistance diagnosis and inform product development.

Laboratory colonies were established from ticks collected in Eldorado do Sul (RS) and Seropédica (RJ). Genetic analysis (12S rRNA sequencing) identified the RS colony as *R. sanguineus* s.s. and the RJ colony as *R. linnaei*. Susceptibility was assessed using the Larval Packet Test (LPT), which calculated lethal concentrations (LC50, μg/mL): fluralaner (3.66 for *R. sanguineus* s.s. vs. 16.44 for *R. linnaei*), fipronil (7.04 vs. 42.59), and permethrin (228.86 vs. 688.88). *R. sanguineus* s.s. was more susceptible to all compounds, showing 4.5-fold, 6.1-fold, and 3-fold lower LC50 values for fluralaner, fipronil, and permethrin, respectively.

Efficacy trials confirmed the RJ strain's susceptibility to fipronil (42 days) and fluralaner (90 days), indicating no resistance to these drugs. A susceptible *R. sanguineus* strain from Greece was used as a reference for LPT with permethrin, revealing low resistance ratios in RS (3-fold) and RJ (2.2-fold) strains. These results were corroborated by the Rapid Tick Exposure Test (RaTexT®). No mutations in the sodium channel gene associated with pyrethroid resistance were detected in any strain.

Interspecific differences in acaricide susceptibility emphasize the importance of broad species control strategies. This study provides essential baselines for resistance monitoring and supports the development of tick control measures in Brazil.
Funding: FAPUR, CAPES, INCT-EM, CNPq.

OP 045

Laboratory breeding of *Ixodes hexagonus* – a tick breeder`s perspective

H. Dautel[1], M. Jonas[1], M. Grau[1], A. Fuhrmann[1], L. Sawicki[1], N. Pfeiffer[1], C. Dippel[1], K. Büchel[1]
[1]IS Insect Services GmbH, Berlin, Germany

Companies marketing anti tick products require large numbers of ticks, e.g. for veterinary clinical trials to demonstrate sufficient efficacy for product registration. As a tick breeder, we therefore maintain a laboratory colony to provide specific pathogen-free ticks on demand. However, the demand for such ticks is erratic and unpredictable, which presents a challenge to a tick breeder, as large numbers of ticks need to be available for studies within a reasonably short time.

Besides *Ixodes ricinus*, the hedgehog tick, *Ixodes hexagonus*, is the most common tick infesting carnivorous pets such as cats and dogs. In nature, hedgehogs are the primary host of *I. hexagonus* which occur in large parts of Europe and adjacent areas. Because of these host species, *I. hexagonus* can occur in close proximity to humans and is therefore of economic and medical importance. It can maintain enzootic cycles of pathogens, in particular *B. burgdorferi s.l.*, whereby hedgehogs can act as bridging hosts for humans as they are easily parasitised by *I. ricinus* in addition to *I. hexagonus*.

Here, we review what is known about the development of *I. hexagonus*, and our attempts to maintain a laboratory colony. We also discuss strategies for economically rearing the tick in a difficult business environment, where customer requirements are unpredictable. In the long term, artificial feeding of *I. hexagonus* may provide an alternative to avoid the use of laboratory animals for tick breeding.

OP 046

The niche of *Borrelia burgdorferi* s.l. in Europe is predictable and mappable

A. Estrada-Peña[1], *A. Davidson*[2], *J. Davis*[3], *S. Willis*[4], *J. Stark*[4], *P. Kelly*[2]
[1]Ministerio de Salud Humana, Madrid, Spain
[2]Pfizer Inc., New York, NY, United States
[3]Clarivate Analytics, Phoenix, AZ, United States
[4]Pfizer, Cambridge, MA, United States

Environmental niche modeling is a well-supported approach for understanding the factors determining tick distribution. However, modeling the niche of tick-borne pathogens (TBPs) has traditionally relied solely on the distribution of presumed tick vectors. That approach is questionable since drivers of TBPs distribution involve both vectors and reservoirs. We aim to demonstrate that bacteria of the group *Borrelia burgdorferi* have a niche in Europe.

We compiled 23,683 records of 11 species of ticks and 7,144,939 records of 215 genera of vertebrates in the Western Palearctic. Using Species Stacking Distribution Modeling, we jointly modeled the distribution of both ticks and vertebrates using Multivariate Adaptive Regression Splines, Support Vector Machine, and Generalized Additive Models. We analyzed the modeled distributions via Principal Components Analysis to reduce complexity. Data on the prevalence of *B. burgdorferi* s.l. in questing nymphs of *Ixodes ricinus* were compiled to describe the niche defined by environmental, host, and landscape variables.

Statistical analysis revealed that the pathogen's primary reservoirs co-occur together. The distribution of *B. burgdorferi* s.l. (presence/absence) is strongly associated with a cluster of co-occurring vertebrates and the range of *I. ricinus*. The presence of *I. ricinus* alone does not reliably predict *Borrelia* spp. distribution.

The distribution of *B. burgdorferi* s.l. is thus largely shaped by climatic factors impacting both vector and reservoir occurrences. The niche of *Borrelia* spp. in Europe is predictable and mappable, mould by the co-occurrence of key vertebrate species. This framework demonstrates that the occurrence of tick-borne pathogens can be realistically mapped.

Efforts to predict the prevalence of *B. burgdorferi* s.l. in questing nymphal ticks were unsuccessful, suggesting that such dynamics operate on a local scale, beyond the resolution of this study.

OP 047

The spatial range of *Borrelia burgdorferi* s.l. in Europe under a future scenario of +2ºC

P. Kelly[1], *A. Davidson*[1], *J. Davis*[2], *S. Willis*[3], *J. Stark*[3], *A. Estrada-Peña*[4]
[1]Pfizer Inc., New York, NY, United States
[2]Clarivate Analytics, Phoenix, AZ, United States
[3]Pfizer, Cambridge, MA, United States
[4]Ministerio de Salud Humana, Madrid, Spain

Species distribution modeling has been used to map the expected range of ticks or to project the built models into climate frameworks. However, the concept has never addressed simultaneously the distribution of ticks and vertebrate reservoirs of tick-borne pathogens. This study aims to pinpointing the expected changes of the niche of *Borrelia burgdorferi* (Bb) in Europe, under a climate scenario of +2ºC, using a previously developed framework.

We compiled 23,683 records of 11 species of ticks and 7,144,939 records of 215 genera of vertebrates in the Western Palearctic. Using Species Stacking Distribution Modeling, we modeled the joint distribution of both ticks and vertebrates, using a set of current temperature and water vapor deficit, evaluating tick-reservoir contact rates *versus* actual presence of Bb. We projected the models into a climate scenario of +2ºC.

The results revealed a tight co-occurrence of the community of vertebrates involved in the transmission of Bb, and occurrence of Bb was found to be impacted by the climate scenario. The interactions among reservoirs and the vector are expected to decrease in parts of central Europe, with large incertitude in southern and northern fringes. The climate scenario constrains the occurrence of Bb in southern regions by the expected decrease of *Ixodes ricinus*, despite the presence of suitable reservoirs. However, an increase of the range of Bb is expected in large territories of the Northern Europe.

The niche of Bb in Europe is both predictable and mappable when communities of co-occurring reservoirs and the tick vector are incorporated as explanatory variables in modeling, as dependent of environmental changes. We found that the trends of the climate would deeply affect the circulation of the pathogen in its southern distribution range, also detecting patchy areas of high probability of occurrence in Central Europe and Northern countries.

OP 048

Insights into outbreaks of *Rhipicephalus microplus* ticks in the cattle tick-free zone in Southern Brazil

J. Reck[1], *G. Gonchoroski*[2], *M. Pereira*[3], *C. Trein*[4], *G. Klafke*[1]
[1]IPVDF, Parasitology Lab, Eldorado do Sul, RS, Brazil
[2]UFRGS, Zoology Department, Porto Alegre, RS, Brazil
[3]Municipal Agriculture Department, Gravatai, RS, Brazil
[4]State Agriculture Department, Santa Vitoria do Palmar, RS, Brazil

The cattle tick, *Rhipicephalus microplus*, is a highly invasive parasite infesting bovines across vast regions of the Americas, Asia, and Africa. In Brazil, it threatens cattle production, causing estimated losses of billions of dollars annually. The parasite is widespread in Brazil, except for a tick-free zone covering approximately 5,500 km^2 in two southernmost municipalities of Rio Grande do Sul State. This tick-free area, south of latitude 32°S, is ecologically unfavorable for *R. microplus*. It borders Uruguay to the south, is separated from the rest of Brazil by a wildlife refuge to the north and is bounded by water bodies to the east and west. Since 1951, the state veterinary service has regulated cattle entry into this zone. Despite these measures, sporadic cattle tick outbreaks occur within the tick-free zone. This study investigated border and buffer zones, identifying contributing factors. Monthly farm visits over two years recorded seven outbreaks. Three outbreaks were linked to farmers illegally grazing cattle in the northern wildlife refuge, where roaming cattle from both tick-infested (north) and tick-free (south) areas were observed. Two outbreaks followed the legal transport of cattle treated with acaricides and verified by state officers, but resistance to fluazuron and organophosphate-pyrethroid mixtures likely allowed immature ticks to evade detection. Another outbreak was tied to tick-infested horses returning from a rodeo, and one to potential illegal cattle movement. The large population of invasive chital deer is under investigation for its role in tick dispersion. These findings highlight the challenges of maintaining tick-free areas amid acaricide resistance and non-compliant practices. Results will inform efforts to enhance surveillance and strengthen management of Brazil's tick-free zone.

Funding: CNPq, FAPERGS, INCT.

OP 049

Structural analysis of Bm86-homolog anti-tick vaccine antigens

E. Jacobs[1,2], *I. Echeverria*[3], *P. Upla*[1], *P. Fridy*[1], *P. D. Olinares*[1], *M. Rout*[1], *A. Sali*[3,1], *B. Chait*[1]
[1]Rockefeller University, New York, NY, United States
[2]St. John's University, Chemistry, Queens, NY, United States
[3]University of California, Cellular and Molecular Pharmacology, San Francisco, CA, United States

Ticks present a significant threat to public health. Ticks are responsible for more human disease transmission than any other vector except for mosquitoes. Ticks also levy a significant economic toll on livestock. A commercial veterinary vaccine was produced that protects cattle from ticks and the tick-borne pathogens that cause bovine anaplasmosis and babesiosis. Although significant differences exist in the host-parasite life cycle between tick species, this successful vaccine provides proof of principle that human anti-tick vaccines that block disease transmission may be possible. The vaccine targets a single antigen, Bm86, which is a glycoprotein expressed in the midgut of the cattle tick *Rhipicephalus* (formerly *Boophilus*) *microplus*. This protein is poorly characterized. Here, we report the characterization of recombinantly expressed Bm86 and three homologs: Ba86, from a closely related species of cattle tick, and Is86-1 and Is86-2 from *Ixodes scapularis*, a tick responsible for transmission of Lyme and other diseases in North America. Using techniques including negative stain electron microscopy, chemical crosslinking mass spectrometry and native mass spectrometry, small angle x-ray scattering, and integrative modeling, we report preliminary structures for the proteins. We have also mapped the glycosylation sites via mass spectrometry and found that the antigens from *I. scapularis* bear roughly twice both the number of glycosylated sites and bulk glycosylation than do their counterparts from the cattle ticks. This may bear relevance to their ability to function as vaccine antigens due to the phenomenon of glycan shielding, which has previously been characterized mainly in viruses. Initial experiments utilizing these homologs to immunize llamas reveal that while a robust antibody response to all antigens is elicited, the titer of antibodies produced with the cattle tick antigens is significantly higher than that of their *Ixodes* counterparts.

OP 050

Molecular characterisation of *Theileria* species in bovine theileriosis in Zimbabwe

V. Pinarello Kleiber[1,2], E. Waniwa[3], M. Mutseekwa[4], T. Nemaungwe[3], D. Wilkinson[2,5]
E. Kupahwana[6], T. Chikaka[6], M. Bourgarel[2,7], D. Pfukenyi[8], T. Hove[9], P. V. Makaya[3], L. Guerrini[2,10]
H. De Nys[1,2]
[1]CIRAD, UMR ASTRE, Harare, Zimbabwe
[2]CIRAD, ASTRE, CIRAD, INRAE, Université de Montpellier, Montpellier, France
[3]Central Veterinary Laboratory, Department of Veterinary Technical Services, Ministry of Lands, Agriculture, Fisheries Water and Rural Development, Harare, Zimbabwe
[4]University of Zimbabwe, Department of Biological Sciences and Ecology, Faculty of Veterinary Science, Harare, Zimbabwe
[5]CIRAD, UMR ASTRE, Sainte-Clotilde, RÉunion
[6]Ministry of Lands, Agriculture, Fisheries, Water, and Rural Development, Department of Veterinary Field Services, Harare, Zimbabwe
[7]Universidade Eduardo Mondlane, Faculdade de Veterinaria, Maputo, Mozambique
[8]Botswana University of Agriculture and Natural Resources, Faculty of Animal and Veterinary Sciences, Private Bag 0027, Gaborone, Botswana
[9]University of Zimbabwe, Department of Veterinary Pathobiology, Faculty of Veterinary Science, Harare, Zimbabwe
[10]CIRAD, UMR ASTRE, Montpellier, France

Several species of the tick-transmitted apicomplexans of the genus *Theileria* infect cattle and wildlife in eastern and southern Africa. *Theileria* (*T.*) *parva*, the most important economically, is the cause of a disease that is a heavy burden for livestock production in Zimbabwe. In recent years, the spatio-temporal patterns of theileriosis outbreaks have changed. Investigating the causative *Theileria* species and strains is key to understanding the infection process and adapting control measures. Samples were collected from suspected bovine theileriosis cases (n=158) in three provinces from November 2020 to April 2021. Frozen blood samples (n= 147) and organs (n=22) were tested by a combination of pan-*Theileria* 18S rRNA PCR, specific *T. parva* PCR and Sanger sequencing to determine the *Theileria* species present. *Theileria* was detected in 83.3% of the sampled clinical cases. *Theileria parva* (63%), *T. velifera* (24%), *T. taurotragi* (2.3%), *T. mutans* (2.3%), and co-infections (3.1%) were detected in the positive samples. To characterise the *T. parva* strains, we sequenced the sporozoite surface antigen *p67* and the CD8+ antigens *Tp2* from 44 and 23 of the *T. parva* positive samples, respectively. Phylogenetic analysis showed that the *T. parva* present was cattle-derived, closely related to the Kenyan Muguga isolate which is part of the widely used *T. parva* vaccine cocktail. Dried blood spots (n=145) and faeces (n=87) were also screened by the 18S rRNA PCR to evaluate their suitability in *Theileria* diagnosis. Sensitivity was less in dried blood spots (59.3%) and faeces (12.7%) than frozen blood, whilst the specificity for blood spots and faeces was 64% and 85.7%, respectively. The use of non-invasive samples such as faeces can however be suitable to study the circulation of *Theileria* at population level, e.g. in a wild animal population. Overall, our data contribute to the knowledge of *Theileria* species circulating in Zimbabwe, informing control strategies and guiding future research.

OP 051

Genomic characterisation of UK *Anaplasma phagocytophilum* strains and optimisation of metagenomic enrichment protocols for whole genome sequencing of *A. phagocytophilum* directly from infected tissues

R. Birtles[1], *S. Brierley*[1], *I. Goodhead*[1], *K. Bown*[1], *L. Bell-Sakyi*[2], *J. J. Khoo*[2], *A. Darby*[2], *A. Al-Khafaji*[2] *B. Makepeace*[2]
[1]University of Salford, School of Science, Engineering and Environment, Salford, United Kingdom
[2]University of Liverpool, Institute of Infection, Veterinary and Ecological Sciences, Liverpool, United Kingdom

Anaplasma phagocytophilum is a tick-borne pathogen of established veterinary importance in Europe and emerging medical importance across temperate regions of the northern hemisphere. Study of the biology and ecology of *A. phagocytophilum* has long been hindered by its fastidious nature that makes its isolation in the laboratory very challenging; to date, only about 30 isolates have been achieved. In this study, we obtained whole genome sequences from all seven UK isolates currently available then used these data to develop strategies for whole genome sequencing of *A. phagocytophilum* directly from infected tissues, thereby circumventing the need for *in vitro* culture. We tested a range of enrichment strategies encompassing differential lysis (Molzym), CpG methylation depletion (NEB), biotinylated RNA bait capture (Agilent SureSelect), and adaptive sampling (ONT) using an infected deer spleen. An optimised approach yielded the majority of the *A. phagocytophilum* genome and application of this approach to blood and spleen from other infected hosts also yielded considerable whole genome sequence data. Thus, the study generated the first complete UK *A. phagocytophilum* genomes, providing insights into genomic diversity and phylogenetic relationships. Optimised enrichment strategies were developed for high-resolution metagenomic sequencing, overcoming challenges posed by low bacterial loads and complex metagenomic samples. Continued genomic characterisation is crucial for understanding the drivers of host specificity, zoonotic potential, and epidemiological dynamics within this diverse tick-borne parasite.

OP 052

RoaM affects the transcription of several putative effectors in *Rickettsia rickettsii* governing the transition to a cellular program of active spreading

A. Nock[1], *T. Clark*[2], *S. Anzick*[3], *C. Martens*[3], *T. Hackstadt*[2]
[1]Texas A&M College of Medicine, Microbial Pathogenesis and Immunology, Bryan, TX, United States
[2]National Institute of Allergy and Infectious Diseases, Host-Parasite Interactions Section, Hamilton, MT, United States
[3]National Institute of Allergy and Infectious Diseases, Research Technologies Branch, Hamilton, MT, United States

Rickettsia rickettsii is an obligate intracellular pathogen causing Rocky Mountain spotted fever, a high morbidity tick-borne disease. In the natural environment, the bacterium must navigate the transition from its arthropod vector to a vertebrate host, and back. Once *R. rickettsii* has invaded a host cell, it can spread from cell to cell by polymerizing actin filaments to propel the bacterium into adjacent cells. While a strategy of aggressive spreading might be necessary in the vertebrate to achieve horizontal transmission of *R. rickettsii* between co-feeding ticks, unchecked spread within the vector could ultimately cause death of the arthropod vector and become a dead end for the bacterium. We previously showed that production of the rickettsial protein RoaM (Regulator of Actin-based Motility) negatively regulates the production of actin tails and its abrogation induces hyper-spreading behavior in many laboratory-adapted strains of *R. rickettsii*.

Using *R. rickettsii* strains derived from the virulent Sheila Smith strain that express varying levels of *roaM*, an RNA-seq experiment was performed. This revealed that *roaM*-deficient strains upregulate at least seven hypothetical proteins which may link the regulatory effects of RoaM to the phenotypic effect on motility. Among the genes regulated is the effector RarP2 which disrupts host cell signaling by dispersing the trans-Golgi. RNA-seq results were confirmed by RT-qPCR. Two of the hypothetical proteins were shown to be secreted via fusion to a glycogen synthase kinase beta tag, which when phosphorylated reveals exposure to the host-cell cytosol. Additionally, growth assays in a tick explant model showed that hyper-spreading strains multiply to a higher number by four days post infection. Taken together, these data support the hypothesis that RoaM affects transcriptional regulation, down-regulating rickettsial genes important in the mammalian host but detrimental in the tick vector.

OP 053

Using machine learning to discover novel tick repellents and acaricides

M. Vlot[1], *M. Vos*[1], *R. Henderson*[1], *J. Konijnenburg*[1], *K. Dechering*[1]
[1]TropIQ Health Sciences, Nijmegen, Netherlands

Tick-borne diseases are a growing threat to human and animal health, necessitating innovative strategies to enhance vector control and deepen our understanding of pathogen transmission. At TropIQ Health Sciences, we have developed a multidisciplinary approach that leverages machine learning, computer vision, and advanced laboratory automation to revolutionize tick-borne disease research. This strategy focuses on the discovery of novel repellents and acaricides, alongside cutting-edge assay development to study disease transmission.

Our machine learning models have proven highly successful at identifying novel tick repellents. By digitizing thousands of repellency and acaricide activity measurements and training graph neural networks (GNNs), we developed a predictive platform for molecule discovery. This approach identified highly effective tick repellents, including compounds outperforming DEET, and achieved a 57% hit rate for repellents and a 6% hit rate for acaricides. Follow-up screenings of analogous compounds further expanded the list of active tick repellents. Additionally, computer vision-based analysis of tick movement has provided insights into the mechanisms of action of repellents, distinguishing between contact irritants and spatial repellents.

Complementing our discovery pipeline, we are advancing automation in tick membrane feeding assays to study disease transmission dynamics, including *Borrelia* spp. transmission. Robotic arms and custom-designed 3D-printed components have enabled high-throughput membrane feeding systems, providing a scalable and reliable platform for studying tick-pathogen interactions. Our integrated approach demonstrates how a data-driven approach can significantly enhance the speed and efficiency of tick-borne disease research. By applying these state-of-the-art technologies, TropIQ has set a precedent for how small R&D labs can make impactful contributions to combating vector-borne diseases.

OP 054

Characterization of secreted *Theileria annulata* effector protein Ta9

S. Tajeri[1,2], *J. Kinnaird*[3], *K. Elati*[1,2], *B. Shiels*[3], *G. Langsley*[4], *A. M. Nijhof*[1,2]
[1]Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany
[2]Freie Universität Berlin, Veterinary Centre for Resistance Research, Berlin, Germany
[3]University of Glasgow, School of Biodiversity, One Health and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, Glasgow, United Kingdom
[4]Université Paris Descartes - Sorbonne Paris Cité, Laboratoire de Biologie Comparative Des Apicomplexes, Faculté de Médecine, Paris, France

In tropical theileriosis of cattle, the major driver of pathology is the transformation of host macrophages by the intracellular apicomplexan parasite *Theileria annulata*. In *Theileria*-transformed leukocytes several oncogene-associated signaling pathways, including Activator Protein 1 (AP-1) and NF-kB, are constitutively activated in a parasite-dependent manner. The proto-oncogene haematopoetic cell kinase (Hck) of the Src family of non-receptor tyrosine kinases is constitutively active in *T. parva*-transformed B cells and contributes to AP-1-driven transcription (1). Ta9 is a *T. annulata* secreted protein that we have shown capable of stimulating AP-1-driven transcription (2) raising the possibility that Ta9 might do so by augmenting Hck signaling. Using two independent anti-Hck antibodies, we now demonstrate co-localization of Ta9 with the active form of Hck in/on cytoplasmic vesicle-like structures. These vesicle-like structures were confirmed to be mitochondria. Expression of full-length GFP-tagged Ta9 in bovine macrophages or mouse fibroblasts (3T3 cells) results in the appearance of numerous spike-like membrane protrusions (“hairy phenotype”) and dorsal ruffles. In addition, Ta9 upregulates Hck transcripts (RNA-seq and qRT-PCR) and protein levels (IFA) in bovine macrophages. These results are consistent with Ta9 potentially binding to and activating Hck and treatment of *T. annulata*-infected macrophages with a selective Hck inhibitor (A419219) negatively impacted on the parasite-dependent transformed phenotype, as estimated by cell proliferation and soft agar colony formation assays. The mitochondrial localization of Hck in macrophages is novel and therefore requires further exploration.

Keywords: *Theileria annulata*, leukocyte transformation, Hck kinase signaling, proto-oncogene

1. *Baumgartner et al. 2003,* Blood.
2. *Unlu et al. 2018,* PLOS ONE.

OP 055

Moving beyond the 18s rRNA gene as a diagnostic target for *Babesia microroti*

T. F. Rounsville Jr.[1], *A. M. Bryant*[1], *A. G. Marini*[2], *O. N. Choi*[1], *J. S. Nugent*[1], *M. McAuliffe*[3], *G. M. Dill*[1]
[1]University of Maine, Cooperative Extension, Orono, MN, United States
[2]University of Maine, School of Biology & Ecology, Orono, MN, United States
[3]University of Maine, College of Earth, Life, & Health Sciences, Orono, MN, United States

Human babesiosis is a zoonotic disease caused by an infection of an intracellular apicomplexan parasite of the genus *Babesia*. In the United States, disease transmission is most frequently caused by the bite of an *Ixodes scapularis* tick infected with *Babesia microti*. In recent years, the case counts of human babesiosis continue to increase, as does the geographic distribution of these pathogens. Passive tick surveillance is often used to track the range and prevalence of *B. microti*; however, a related apicomplexan species, *B. odocoilei*, may be confounding some molecular testing methods in locations where these two species co-occur. Over the course of five years we completed thousands of qPCR tests targeting the 18S rRNA gene of *B. microti* in *I. scapularis* ticks collected passively in the U.S. state of Maine. During this testing we noticed an anomalously large number of samples that were positive with low concentrations of *B. microti*. Further work using Oxford Nanopore sequencing on the 18S rRNA gene revealed that many samples testing positive for low levels of *B. microti* were actually infected with *B. odocoilei*. In addition, some samples that were molecularly identified by sequencing a roughly 5kb portion of the mitochondrion had 18S rRNA genes belonging to the other species, which is possible evidence of the sharing of genes between *B. microti* and *B. odocoilei*. In order to improve the diagnostic accuracy of our tests for *B. microti*, we developed two new qPCR assays that target the mitochondrial DNA of both *B. microti* and *B. odocoilei*, respectively. After more than 3,000 tests using these new assays, we have concluded that mitochondrial DNA offers improved diagnostic specificity for qPCR assays of *Babesia* spp. than the more frequently used 18S rRNA gene.

OP 056

Babesiosis as an emerging concern in the Mid-Atlantic United States – Autochthonous human babesiosis cases and *Babesia microti* (Piroplasmida: Babesiidae) in *Ixodes scapularis* (Acari: Ixodidae) and *Ixodes keiransi* (Acari: Ixodidae) ticks from Delaware, Maryland, Virginia and West Virginia, 2009–2024

R. Nadolny[1], *E. Stromdahl*[1], *K. Feldman*[2], *Z. Bement*[3], *M. Buoni*[4], *A. Kennedy*[5], *J. Bernick*[6]
R. J. Brinkerhoff[7], *J. Broyhill*[6], *D. Crum*[2], *A. DaSilva*[8,9], *E. Dotseth*[10], *L. Flammia*[6], *K. Girone*[6]
D. Gaines[6], *A. Phan*[3], *B. Pritt*[11], *H. Rutz*[2], *S. B. Wee*[2], *H. Gaff*[3], *W. Hynes*[3]
[1]Defense Centers for Public Health - Aberdeen, Vector-Borne Disease Branch, Edgewood, MD, United States
[2]Maryland Department of Health, Baltimore, MD, United States
[3]Old Dominion University, Biological Sciences, Norfolk, VA, United States
[4]Delaware Technical Community College, Georgetown, DE, United States
[5]Delaware Department of Natural Resources and Environmental Control, Newark, DE, United States
[6]Virginia Department of Health, Richmond, VA, United States
[7]University of Richmond, Richmond, VA, United States
[8]US Food & Drug Administration, Laurel, MD, United States
[9]Centers for Disease Control and Prevention, Atlanta, GA, United States
[10]West Virginia Department of Health, Charleston, WV, United States
[11]Mayo Clinic, Rochester, MN, United States

The range of *Babesia microti* (Franca, 1910)-infected ticks is expanding, resulting in locally-acquired human babesiosis cases occurring in new areas of the United States: Maryland (2009), Virginia (2016) and West Virginia (2017). We collected host-seeking ticks from old fields, ecotones and forested habitats in Delaware, Maryland, and Virginia, 2010-2023. *Ixodes scapularis* Say, the tick vector of babesiosis, was captured in all three states. PCR revealed *B. microti* in 2.7% (36/1310) of *I. scapularis* from the three states, with sites ranging from <1% to 12.5% infected. The first *B. microti*-positive *I. scapularis* was collected in Northampton County, Virginia in 2012. Of the *B. microti*-infected ticks, 50% (18/36) were coinfected with *Borrelia burgdorferi* and one was triple-infected with *B. microti, B. burgdorferi* and *Anaplasma phagocytophilum*. We tested a subset of these tick samples for *Babesia duncani*; none were positive. We also collected *Ixodes keiransi* Beati, Nava, Venzal, and Guglielmone ticks from our study sites in Virginia and Delaware and found *B. microti* and *B. burgdorferi* in ticks from Virginia; those from Delaware were uninfected. To our knowledge, these are the first reports of *B. microti*-positive *I. scapularis* and *I. keiransi* from Delaware, Maryland and Virginia. *Ixodes keiransi* ticks rarely bite humans but are involved in the maintenance and spread of human pathogens when sympatric with *I. scapularis*. Jurisdictions in these mid-Atlantic states should expect babesiosis cases and Lyme-babesiosis coinfections, and healthcare providers should consider these tick-borne infections as part of the differential diagnosis.

OP 057

The concept of natural transmission of TBE virus revisited

G. Dobler[1], *L. Chitimia-Dobler*[2], *M. Pfeffer*[3]
[1]Bundeswehr Institute of Microbiology, Munich, Germany
[2]Fraunhofer Institute of Immunology, Infection and Pandemic Research, Penzberg, Germany
[3]Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany

Tick-borne encephalitis (TBE) is the most important tick-borne viral disease in Europe and Asia. The TBE virus is circulating in a natural transmission cycle between ticks (vectors) and certain small mammals (reservoir hosts). Historically, the transmission of an arbovirus is via the bite of the vector and the uptake of the pathogen by feeding vector ticks via viremic blood. Although this fundamental knowledge is well accepted for TBE virus, many details of the transmission cycle in tick-borne viruses are still obscure. The fact that only a small portion of ticks in a natural focus is infected and only a low percentage of small mammals is found to be infected with the TBE virus caused doubts about the long-existing ideas on the importance of viremic transmission in analogy to mosquito-borne arboviruses.

These doubts were strengthened when in the late 1980s and 1990s non-viremic transmission of tick-borne viruses, among them also TBE virus, via co-feeding of ticks was detected. Accepting this new mode of transmission, there was common agreement among many tick virologists that this may explain how low tick infection rates may maintain the virus transmission cycle. However, so far, no field evidence on the importance of non-viremic transmission by co-feeding ticks has been detected. All available direct evidence resulted from laboratory experiments or from indirect evidence in the field.

In a recent study over 5 years a TBE virus natural focus was studied and the virus prevalences in ticks and infection rates in small mammals were monitored. The data do not show any evidence for the importance of co-feeding and or non-viremic transmission. The results show a constant infection rate of 0.5 to 5% in nymphs and adult *Ixodes ricinus* and a constant seroprevalence in bank voles of about 20% over the whole study period. However, we detected some unusual features in infected bank voles which, together with some historical data about the distribution of infected ticks in the field, caused us to postulate a new mode of transmission cycle for TBE virus in nature. Applying this new mode of transmission, many of the so far unexplained features of transmission of TBE virus in the field may now become clear. It has to be seen whether this new concept may also apply to other tick-borne viruses.

PP 047

Recombinant Spm2 protein for serological diagnosis of cattle theileriosis

A. Ryskeldina[1], *N. Tursunbay*[1], *A. Shevtsov*[1], *A. Dauletov*[1], *M. Kuibagarov*[1]
[1]National center for biotechnology, Laboratory of Applied Genetics, Astana, Kazakhstan

Theileria annulata infection in cattle causes significant economic losses in livestock production in many Central Asian countries, including the southern regions of Kazakhstan. This study aimed to obtain a recombinant sporozoite and macroschizont gene 2 (Spm2) protein from *T. annulata* and investigate its potential use as an antigen in an indirect enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of bovine theileriosis.

The Spm2 protein was cloned and expressed in *E. coli* for further analysis. For recombinant expression, a fragment of the gene corresponding to amino acid residues 640 to 850 of the *Spm2* gene (XP_952546.1) was used. The gene fragment was synthesized using the *de novo* gene synthesis method and assembled via nested PCR. The synthesized gene fragment was cloned into the expression plasmid pET28, followed by transformation into the *E. coli* BL21(DE3) strain by inducing with IPTG at 18°C overnight, incorporating a 6-His tag and restriction sites.

The Spm2 protein, derived from the soluble fraction, was purified in a single step using Ni-NTA affinity column chromatography. The bound protein was eluted using a linear gradient of imidazole (50 mM to 250 mM). SDS-PAGE analysis of the purified Spm2 showed the expected protein size of approximately 29 kDa, and mass spectrometry analysis identified the purified protein as a fragment of the Spm2 of *T. annulata*.

The purified Spm2 protein was used as an antigen to conduct an indirect ELISA assay. To minimize nonspecific binding, an adsorption buffer containing the lysate of the BL21(DE3) (1000 mcg/ml) expression strain was used as the plasma sample dilution buffer. Initial testing of 72 field plasma samples from cattle revealed that the results of the indirect ELISA using Spm2 as an antigen closely matched those of *T. annulata* PCR (kappa value: 0.85). The relative sensitivity and specificity of the ELISA were 92.7% and 80%, respectively, using PCR as the reference.

PP 048

Regional differences in Tick-Borne Encephalitis diagnostics across Germany – a three-year multicenter study

<u>J. Leifheit</u>[1,2], K. Lehmann[2], C. Malerczyk[1], C. von Eiff[1], K. Becker[2]
[1]Pfizer Pharma GmbH, Berlin, Germany
[2]Friedrich Loeffler-Institut, Universitätsmedizin Greifswald, Greifswald, Germany

Background and study aim

Tick-borne encephalitis (TBE) is a viral arthropod infection of the central nervous system (CNS) that can cause severe neurological effects with long-term consequences. While most cases in Germany occur in southern endemic areas, northern regions have reported autochthonous cases, serological evidence in animals, and new virus reservoirs. This study hypothesized that TBE in non-risk areas may be underestimated and aimed to examine regional differences in diagnostic rates and positive sample proportions.

Material and methods

In this multicenter study, data were collected from ten laboratories over a period of three years. Cerebrospinal fluid (CSF) samples with suspected CNS infection and all sera for which Lyme borreliosis was requested were included. The TBE diagnostic rates were evaluated relative to general CNS infection diagnostics and suspected Lyme borreliosis, regionally stratified. Assuming Lyme borreliosis and CNS infections are evenly distributed across Germany, diagnostic rates were used as a denominator for comparability across laboratory catchment areas.

Results and discussion

Preliminary evaluations were conducted for three laboratories each within and outside endemic areas. The TBE diagnostic rate (CSF/serum samples tested for TBE relative to CSF samples with suspected CNS infection) was notably lower in non-risk areas (4.7%) compared to risk areas (7.2%). The TBE diagnostic rate in sera analyzed for Lyme borreliosis was considerably higher in risk areas (18.1%) compared to non-risk areas (4.1%).

The lower TBE diagnostic rates in non-risk areas suggest that TBE infections are underdiagnosed in these regions, potentially resulting in undetected cases. Therefore, in cases of suspected meningoencephalitis, specific TBE diagnostics should also be performed in non-risk areas. An expanded definition of risk areas, e.g. taking into account regional TBE virus prevalence, could help raise risk awareness among physicians and patients.

PP 049

Discrepancies in widely used serological methods to detect *Borrelia* infections may cause missed Lyme diagnoses

E. Volokhina[1], *A. Stemerding*[2], *Y. Smits*[1], *J. Lankhof*[1], *M. Kouwijzer*[1], *A. Garritsen*[1]
[1]InnatOss Laboratories B.V., Oss, Netherlands
[2]Deventer Hospital, Deventer, Netherlands

The accurate diagnosis of Lyme disease, caused by *Borrelia burgdorferi*, is critical for effective treatment and prevention of disabling long-term effects. This study investigates discrepancies in serological testing methods for *Borrelia* infections, focusing on the performance of different assays in annual Lyme screening for high-risk populations. We compared the results of two-tier protocols, including ELISA assays from EUROIMMUN, ZEUS, Diasorin, and Serion. Our findings reveal significant variability in test sensitivity, with EUROIMMUN and ZEUS ELISAs detecting more positive cases compared to Diasorin CLIA and Serion ELISA. Notably, 19% of recent *Borrelia* infections were missed due to test selection, highlighting the clinical implications of these discrepancies. Case studies illustrate the potential for misdiagnosis and delayed treatment, emphasizing the need for standardized and optimized testing protocols. We recommend the adoption of more sensitive and comprehensive methods, and the practice of comparing current results with previous samples from the same individual to improve diagnostic accuracy and patient outcomes.

PP 050

Improvement of the serodiagnosis of tick- and louse-borne relapsing fever

F. Reyer[1], *V. Fingerle*[2], *A. Latz*[3], *U. Steinhoff*[4], *S. Besier*[1], *V. A. J. Kempf*[1], *P. Kraiczy*[1]
[1]Goethe-University Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt a.M., Germany
[2]German National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany
[3]Gold Standard Diagnostics GmbH, Dietzenbach, Germany
[4]Philipps-University Marburg, Institute for Medical Microbiology and Hygiene, Marburg, Germany

Background

Tick- and Louse-borne relapsing fever (RF) are caused by diverse *Borrelia* species. The recurrent episodes of life-threatening fever are responsible for these neglected infectious diseases. The diagnosis of relapsing fever is challenging, especially in impoverished countries. Microscopy of blood smears is still the gold standard even PCR-based methods have already been described but not implemented in most African countries. Recently, we developed two immunoassays, ELISA and Line blot, for the serodiagnosis of Louse-borne RF. This study deals with the improvement of both immunoassays.

Methods

Immunoreactive antigens originating from *Borrelia recurrentis* were used in different combinations with GlpQ and CihC-N already implemented in these immunoassays. To identify promising candidates and antigen combination resulting in a low cross-reactivity and a high specificity and sensitivity, a broad serum panel including samples from RF patients, syphilis, leptospirosis, malaria, leishmaniasis, tuberculosis, HIV, CMV, and EBV as well as healthy blood donors was utilized.

Results

The data of the IgM Lineblot revealed an increase in sensitivity by 42.9 % (reaching 92.9 %) with a slight reduction of the specificity of approximately 2.2 % (dropping to 87.6 %). In terms of IgG, the specificity was improved by 11.7 % (rising to 90.5 %) without a loss in sensitivity.

Discussion

By implementing additional borrelial antigens with high specificity, we were able to improve the Line blot immunoassay which is applicable for the serodiagnosis of Tick-borne RF. Ongoing studies focus on the improvement of the ELISA screening test. Preliminary data suggested an increase in specificity of 10 % (exceeding 90 %) for the IgM ELISA. These advancements, compared to the previous assays, have led to the development of two reliable diagnostic methods, which simplify the diagnostic process and may enhance the awareness of RF in different African countries where RF circulates.

PP 051

A new method for rapid and accurate detection of Borreliaceae DNA based on *flaB* gene sequences

A. Taylor[1]*, A. Trzebny*[1]*, J. Michalik*[2]*, M. Dabert*[1]
[1]Uniwersytet im. Adama Mickiewicza w Poznaniu, Molecular Biology Techniques Laboratory, Poznań, Poland
[2]Uniwersytet im. Adama Mickiewicza w Poznaniu, Department of Animal Morphology, Poznań, Poland

The *Ixodes ricinus* tick is the main vector of tick-borne diseases in Europe. It is responsible for the transmission of various pathogens, most notably Lyme borreliosis agents, i.e., *Borreliella* spirochetes. In this study, we aim to present a new primer set "Abi-*flaB*" for the efficient detection of *Borreliella* and *Borrelia* spp. DNA in DNA extracts. Our method is based on amplification of a short region (249 bp) of the *flaB* gene, followed by next-generation sequencing (NGS) for a rapid, sensitive, accurate and high-throughput screening of ticks. The primer set was tested in silico to ensure that the sequence of the short amplicon enables differentiation of all Borreliaceae flagellin sequences available in GenBank. The Abi-*flaB* primers were tested by screening 1088 *I. ricinus* DNA isolates, which were grouped into 94 DNA pools. The results were compared to a conventional nested PCR amplification of the *flaB* region, and to 16S rRNA gene profiling. Out of 94 DNA pools, 83 were found positive by sh *flaB*, 58 by *flaB* nested PCR, and 55 by 16S rRNA gene profiling, further highlighting the superiority of targeting the *flaB* gene and the sensitivity of NGS.

PP 053

Comparison of the diagnostic performance of PCR kits for detecting tick-borne pathogens in tick specimens

S. Sadangi[1]*, M. L. Espinel Ramos*[2]*, T. Falkenberg*[1]*, M. Parcina*[3]
[1]Universitätsklinikum Bonn, Institut für Hygiene und Public Health, GeoHealth Centre, Bonn, Germany
[2]University of Bonn, Zentrum für Entwicklungsforschung (ZEF), Bonn, Germany
[3]Institute für Medizinische Mikrobiologie, Immunologie und Parasitologie, Bonn, Germany

Early detection of tick-borne diseases using simple and cost-effective diagnostic tools in medical practice is crucial. This study evaluates the diagnostic efficiency of three commercially available PCR kits: Novaplex™ Tick-borne Disease Expanded Assay, RealLine *Borrelia burgdorferi* s.l. (Str-Format) kit, and *alphaCube Borrelia/Rickettsia* Assay for detecting *Borrelia (B.) burgdorferi* s.l.*, B. miyamotoi* and *Rickettsia spp.* and co-infections in tick specimens from the Bonn region of western Germany. A total of 722 tick specimens (101 larval pools, 576 nymphs, and 45 adults) collected from June to October 2022 were examined for the prevalence of these pathogens. Both Bioron and Mikrogen assays exhibited 100% sensitivity (95% CI: 96.55-100%) and 100% specificity (95% CI: 99.29-100%) for detecting *Borrelia burgdorferi s.l.* DNA. However, the Novaplex™ assay, despite its high specificity (100%, 95% CI: 99.29%–100%), showed reduced sensitivity (83.81%, 95% CI: 75.35%–90.28%). The diagnostic efficiency of both Novaplex™ and In-house PCR assay was found to be excellent for the detection of *Borrelia miyamotoi* DNA, with 100% sensitivity (95% CI: 80.49%–100%) and specificity (95% CI: 99.48%–100%). For the detection of *Rickettsia spp.* DNA, while the Novaplex™ assay demonstrated excellent diagnostic efficiency with 98.91% sensitivity (95% CI: 94.09%–99.97%) and 99.84% specificity (95% CI: 99.12%–99.99%), the Mikrogen assay showed 92.39% sensitivity (95% CI: 84.95%–96.89%) and 100% specificity (95% CI: 99.42%–100%). The findings of this research study have critical clinical applications, especially for early diagnosis and management of tick-borne diseases. Future research will expand the evaluation to include other tick-borne infections, such as tick-borne encephalitis virus, *Anaplasma spp*., and *Babesia spp*., with a larger sample size for validation.

PP 054

Detection and isolation of *Francisella tularensis* using recombinant reporter fusion proteins

J. K. Janssen[1], *G. Grass*[1], *H. von Buttlar*[1], *R. Woelfel*[1]
[1]Bundeswehr Institute of Microbiology, Munich, Germany

Francisella tularensis (*F.tul*) causes tularemia in animals and humans. The main vectors of transmission in Germany are ticks. The fast and accurate detection of *F.tul* and its isolation from the environment and subsequent culture is still challenging. In this work, we employed a *F.tul*-specific single-chain variable fragment antibody (scFv) to develop a method for the sensitive and rapid detection of *F.tul* cells and their enrichment and isolation from crushed ticks. For this, synthetic genes of a *F.tul*-specific scFv and a fluorescent protein were fused by PCR-amplification and cloned into the expression vector pASG-105 harboring a Twin-Strep-Tag-epitope. The resulting reporter fusion protein (RFP) was heterologously produced in *Escherichia coli* and purified by affinity chromatography. Binding of the RFP to a range of *Francisella* spp. and other bacteria was assessed by fluorescence microscopy. The identification of *F.tul* cells in the clinical matrices blood and sputum was positively evaluated. Additionally, the RFP was immobilized to magnetic beads. These RFP-loaded magnetic beads were utilized to separate *F.tul* cells from other microbes in crushed ticks. The enriched *F.tul* cells were cultured on agar plates. Next, the isolates were analyzed via fluorescence microscopy with the RFP and by PCR. A newly designed delayed mismatch amplification assay (DMAA) was employed to genotype the new *F.tul* isolates based on specific single nucleotide polymorphisms (SNPs).

All *F.tul* cells were successfully labeled by the RFP; more distantly related bacteria were not. The only non-*F.tul* bacteria labeled were *F. hispaniensis* and *Y. pestis.* (but not other *Yersinia* spp.). Thus, the fast detection of *F.tul* in relevant clinical matrices is possible. Using RFP-loaded magnetic beads we successfully isolated *F.tul* cells from ticks collected in a Munich park. Next, we aim to further improve this method and expand its application to selectively isolate *F.tul* from other environmental samples.

PP 055

Innovative machine learning algorithms to understand the driving forces for tick abundance across Europe

S. Lansdell[1], *A. Zorto*[2], *M. Seto*[1], *E. Negera*[1], *M. S. Sharif*[2], *S. Cutler*[1]
[1]University of East London, Health, Sport and Bioscience, London, United Kingdom
[2]University of East London, Intelligent Technologies Research Group, London, United Kingdom

Ixodes ricinus population expansion across Europe (and their associated tick-borne pathogens) have prompted an urgent need to understand the complex interplay of biotic and abiotic factors driving tick survival, and predict which areas have higher risk levels. Others have used Machine Learning (ML) models to predict tick abundance and determine which factors contribute towards this, but these studies have often been limited in sample size, geographical range, duration and model algorithms used.

To overcome these limitations, we used a large dataset of 27,150 tick occurrence records obtained from 37 European countries over a 20-year period. Each occurrence record was coupled with feature data (temperature, rainfall, vegetation index and land use category). The data were used to train 11 ML models to predict tick abundance, and the success of each model was evaluated using statistical comparison of predicted and actual values. In the second part of our work, we used the Random Forest model and excluded each feature from the analysis individually to determine its impact on predictive accuracy (thus representing the importance of each feature in tick abundance forecasting).

Our results revealed that Linear Regression was best suited to predictive modelling of tick abundance. We also found that land use was the main factor impacting upon tick abundance, with rainfall and temperature playing less of a role.

In summary, this study has identified the best approach to use for accurate tick abundance prediction, which can potentially guide future work (such as abundance modelling for other tick species, or even pathogens). In addition, it has provided valuable insights into the factors most strongly linked to *Ixodes ricinus* presence, which can help to guide tailored intervention strategies to reduce the future burden of tick-borne disease.

PP 056

The Zanzemap project – artificial Intelligence models to forecast vector dynamics in Northern Italy

<u>G. Marini</u>[1], D. Da Re[1], F. Dagostin[1], M. Blaha[1,2], A. Rizzoli[1]
[1]Fondazione Edmund Mach, San Michele all'Adige, Italy
[2]University of Trento, Trento, Italy

The project "ZanZeMap" aims to enhance public health in the Autonomous Province of Trento (Northern Italy) by developing user-friendly maps that indicate the risk of tick and mosquito presence and activity, addressing significant public health challenges posed by vector-borne diseases. Utilizing advanced artificial intelligence (AI) and machine learning techniques, this initiative analyzes detailed climatic and environmental data to predict where and when these arthropods are most active.

Key to this project is the integration of high-resolution climate data, including satellite observations, providing insights into temperature, humidity, and vegetation cover—critical factors for understanding vector habitats and behaviors. The project can forecast changes in vector populations up to two weeks in advance under various climate scenarios, allowing for proactive vector management.

Additionally, field-based vector monitoring will be incorporated to validate the model's forecasts, enhancing the accuracy of vector activity assessments and enabling timely interventions.

The resulting online maps will empower the local population and stakeholders by providing real-time information on vector phenology and activity, facilitating personal protective measures against bites such as using repellents and fostering a collaborative environment in public health initiatives. Ultimately, this project not only aims to improve local vector surveillance but also has the potential for application in diverse geographical contexts facing similar public health challenges exacerbated by climate change. By establishing a robust framework for ongoing data analysis and community involvement, the initiative seeks to enhance public health outcomes and quality of life in the Autonomous Province of Trento and the Alpine area in the future.

PP 057

First insights into pathogen presence and diversity in the Luxembourg's non-native *Dermacentor reticulatus* tick population

P. Reteng[1], *C. Simon*[2], *C. D. Gamio*[1], *A. I. Krawczyk*[1], *A. M. Weigand*[2], *J. M. Hübschen*[1]
[1]Luxembourg Institute of Health, Department of Infection and Immunity, Esch-sur-Alzette, Luxembourg
[2]Musée National d'Histoire Naturelle de Luxembourg, Luxembourg, Luxembourg

Dermacentor reticulatus (Fabricius, 1794) is the second most reported tick species in central Europe (1). In Luxembourg, the first validated occurrence was recorded in 2009 and more findings have been reported ever since, particularly from the south of the country (2). However, there is currently no data on the pathogens present in this tick species in Luxembourg. *Dermacentor reticulatus* ticks were collected between May and June 2023 in southern Luxembourg by flagging and by direct inspection of the vegetation. The tick species was determined with a morphological key and sequencing of the cytochrome *c* oxidase subunit I gene was used when necessary for molecular confirmation. In total, 510 adult *D. reticulatus* were collected. To screen for the presence of pathogens, nucleic acids were extracted from individual ticks and will be tested using real-time PCRs for *Rickettsia* spp., *Babesia* spp., *Anaplasma phagocytophilum*, *Candidatus Neoehrlichia mikurensis*, *Spiroplasma ixodetis*, *Bartonella* spp., *Borrelia burgdorferi s.l.*, and tick-borne encephalitis virus. Positive samples will be further characterized with additional PCR assays. This study provides valuable information on pathogen presence and genetic diversity in the *D. reticulatus* population in Luxembourg.

References:

1. Noll M, Wall R, Makepeace BL, Vineer HR. Distribution of ticks in the Western Palearctic: an updated systematic review (2015–2021). Parasit Vector. 2023;16(1):141.
2. Reye Anna L. Prevalence and Diversity of Tick-Borne Pathogens from Central and Eastern Europe as well as West Africa [Dissertation]. Homburg, Saarland: University of Saarland; 2011.

PP 058

Ixodes ricinus ticks survive flooding

J. Rapp[1]*, A. Springer*[1]*, C. Strube*[1]
[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hanover, Germany

Ixodes ricinus ticks are resilient ectoparasites, capable of surviving adverse conditions, including temporary water submersion, although they typically avoid direct water contact and rely on atmospheric moisture for rehydration. Climate-change induced weather extremes like floods are increasing in frequency and intensity, putting the ticks' adaptability to the test.

Between December 2023 and January 2024, extensive flooding occurred in Lower Saxony, submerging a regularly monitored tick collection site in Hanover for 25 days. Despite this, *I. ricinus* reappeared shortly after the water receded: A questing female was collected 20 days after the flood, and ten days later, an additional 12 ticks were found, suggesting that tick density had returned to pre-flood levels by February 2024.

Soil debris on some ticks suggests that they survived by sheltering in the ground. The rapid recovery of nymphal and adult questing activity indicates that the immediate impact of the flood on tick abundance was minimal. To assess potential long-term consequences, especially regarding the vulnerable larvae and egg stages, further monitoring of the site in comparison to sites unaffected by flooding, is ongoing and will shed light on how extreme weather events may affect *I. ricinus* populations.

PP 059

The survivability of *Rhipicephalus sanguineus* s.s. and *Rh. linnaei* in Germany – a controlled outdoor study

K. Fachet-Lehmann[1], *A. Lindau*[1], *U. Mackenstedt*[1]
[1]University of Hohenheim, Parasitology, Stuttgart, Germany

Rhipicephalus sanguineus sensu lato (s.l.) represents a cosmopolitan tick species complex primarily associated with dogs. Although there are no published endemic occurrences of *R. sanguineus* s.l. in Germany, specimens have been regularly introduced since the 1960s[1]. Since then, it is believed that *Rhipicephalus* can reproduce indoors, but cannot survive outdoors. Imports of *R. sanguineus* s.s., and *R. linnaei* from foreign countries have been introduced regularly with dogs. As climate change leads to milder winters, this raise concerns that these introduced tick species may become established outside of buildings. A one-year outdoor survival study was conducted to assess the viability of all developmental stages (larvae, nymphs, adults) along with egg deposition by engorged females and the hatching of larvae for both introduced *Rhipicephalus* species. Experimental cages containing individuals of both species separately were placed in secure plots at periodic intervals and the number of surviving ticks was recorded weekly. The results revealed that *R. sanguineus* s.s. exhibited longer average survival time than *R. linnaei* at all developmental stages. During the warmer months (Mar. to Nov.), adults survived for 20–44 weeks, nymphs for 7–20 weeks, and larvae for 2–5 weeks. Engorged females from both species successfully laid eggs from which viable larvae hatched, that survived for 10-12 weeks. However, no species was able to survive during the winter period (Nov. to Feb.) for more than 2–4 weeks. These findings indicate that outdoor reproduction is indeed possible, as each stage can endure long enough to find suitable hosts during warmer periods. Although the climatic conditions during the winter of 2023/24 were not sufficient to ensure survival because of wet and freezing conditions, there is a possibility that *R. sanguineus* s.s. could become established in the warmer areas in Germany.

[1] Gothe & Hamel (1973) Zur Ökologie eines deutschen Stammes von *Rhipicephalus sanguineus* (Latreille, 1806) Z Parasitenkunde

PP 060

Tick distribution and phenology across altitudinal gradients in the central Pyrenees, Spain: preliminary results

S. Soares[1], *R. Rodríguez-Pastor*[1], *N. Juárez*[2], *J. Millán*[3,4]
[1]Universidad de Zaragoza, Parasitología y Enfermedades Parasitarias, Zaragoza, Spain
[2]Universidad de Zaragoza, Facultad de Veterinaria, Zaragoza, Spain
[3]Fundación ARAID, Zaragoza, Spain
[4]Instituto Agroalimentario de Aragón-IA2, Zaragoza, Spain

Tick-borne diseases represent a significant public health challenge globally, with ticks transmitting a wide range of zoonotic pathogens. Despite their importance, data on tick ecology, host interactions, and pathogen prevalence in the Pyrenean region remains scarce. The PyrTick Project, supported by the Interreg POCTEFA program, aims to fill this gap by implementing a cross-border, multidisciplinary approach.

Methodology

Systematic field sampling was conducted from May to November 2024 using standardized vegetation flagging to collect questing ticks, covering both forest and pasture habitats. Sampling sites were distributed at 200-meter elevation intervals in five valleys in Aragón (Northern Spain), starting at 600-800 m.a.s.l. and up to 1,600-1,800 m.a.s.l., depending on the valley. In addition, micromammals were captured in spring in three of the valleys, at low, intermediate, and high altitudes, with 20 Sherman traps per elevation for 4 consecutive nights, totaling 60 traps per night. Ticks were morphologically identified.

Results

A total of 1,765 ticks were collected, comprising 967 larvae, 701 nymphs, 54 adult males, and 43 adult females. *Ixodes ricinus* was the dominant species identified, with peak activity recorded at 1,000–1,200 m. Moreover, the number of larvae peaked in August, nymphs in May, and adults in May and June. Vegetation flagging also detected other tick species, including *Ixodes acuminatus*, *Haemaphysalis punctata*, *Dermacentor marginatus*, and *Hyalomma* spp. From host sampling, 86 small mammals were captured, of which 71 were found to host ticks. The captured mammals included: *Apodemus sylvaticus*, *Apodemus flavicollis*, *Clethrionomys glareolus*, *Eliomys quercinus*, *Mus spretus*, and *Crocidura russula*. The most prevalent species in these individuals was *I. ricinus*.

Next Steps

These preliminary results provide the first insights into the distribution and dynamics of tick populations across altitudes and habitats in the Pyrenees.

PP 061

Ticks and tick-borne pathogens in the city park of Greifswald, an urban area of Mecklenburg-Western Pomerania, in 2022

S. Fischer[1], *S. Drewes*[1], *E. Sägert*[1], *C. Silaghi*[1]
[1]Friedrich-Loeffler Institut, Institut für Infektionsmedizin, Greifswald, Germany

Background: Urban areas like city parks are considered a melting pot for contact between ticks, pathogens and their hosts. Therefore, knowledge of tick species and composition are interesting from a public health point of view. Additionally, the prevalence of different pathogens like *Babesia* spp., *Borrelia* spp. and tick-borne encephalitis virus (TBEV) which can be harmful to domestic animals and humans is of great importance.

Method: Questing ticks were collected by the flagging method in the city park of Greifswald, northern Germany, once per month from May to November 2022. All collected ticks were morphologically identified under a stereomicroscope to species level and screened by PCR or real-time PCR for the pathogens *Babesia* spp., *Borrelia* spp., *Anaplasma phagocytophilum* and TBEV.

Results: In total, 530 ticks were collected from May to November 2022. One *Dermacentor reticulatus* male tick and two *Haemaphysalis concinna* nymphs were found, all other ticks represented all developmental stages of *Ixodes ricinus*. All samples were tested negative for TBEV and *Babesia* spp. The prevalences for *Borrelia* spp. in adult female ticks were 10.7% (11/103), in male 4.3% (4/92) and the MIR (minimum infection rate) was 0.87% in nymph pools (2/230). One positive sample was generally defined as *B. burgdorferi s.l.* and others to genospecies belonging to the *B. burgdorferi (s.l.)* group, *B. afzelii* (n=2), *B. garinii* (n=4) and *B. valaisiana* (n=6). Additionally, we detected *B. miyamotoi* (n=6) belonging to the relapsing fever group. For *A. phagocytophilum*, we found a prevalence in adult female ticks of 2.9% (3/103) compared to male ticks 1.1% (1/92). A MIR of 0.87% was registered in nymphs (2/230)

Conclusion: This study shows that different tick species and at least two tick-borne pathogens occurred in the city park of Greifswald in 2022. The risk of transmission of these pathogens to domestic animals or humans can be an important influence on public health.

PP 062

Tick abundance and tick-borne pathogen prevalences in different ecosystems in the Czech Republic in 2024

E. Richtrová[1]*, K. Kybicová*[1]*, J. Navrátil*[1]*, V. Hönig*[2]*, J. Kamiš*[2]*, M. Kulma*[3]*, Z. Vacek*[4]
[1]National Institute of Public Health, National Reference Laboratory for Lyme Borreliosis, Prague, Czech Republic
[2]Biology Centre CAS, Laboratory of Arbovirology, Institute of Parasitology, České Budějovice, Czech Republic
[3]National Institute of Public Health, National Reference Laboratory for Vector Control, Prague, Czech Republic
[4]Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Prague, Czech Republic

Tick-borne bacterial diseases belong to the most widespread vector-borne diseases in Central Europe, including the Czech Republic. The common biotopes for *I. ricinus* are deciduous and mixed forest stands and shrubs with a rich herb layer. Ticks are more abundant at the edges of forests and in areas bordering water sources in a fragmented landscape, however they also colonize suitable urban habitats such as parks, gardens, cemeteries, etc.

The aim of the present study was to determine the abundance of ticks and the prevalence of tick-borne pathogens (*Borrelia burgdorferi* s.l., *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Neoehrlichia mikurensis*, and *Rickettsia* spp.) in the selected urban and forest areas in the Czech Republic.

We have selected 150 forest areas throughout the territory of the Czech Republic. In June 2024, 15,958 ticks were collected by flagging. We also collected 4,032 ticks from 30 small towns evenly distributed across all regions of the Czech Republic in June 2024; and 3,297 from all 13 large regional cities in May 2024.

The highest abundance was observed in large regional cities, 19 ticks per 100 m^2. The average density in forest areas was 16.2 ticks per 100 m^2; and in the small towns 11.7 ticks per 100 m^2. 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. The ticks from forest areas were infected by *B. burgdorferi* s. l. in 10.4% and by *A. phagocytophilum* in 1.8%. The highest prevalence of *B. burgdorferi* s. l., over 30%, was found in small towns.

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 even higher in urban areas than in natural ecosystems.

This study was supported by HADEA - EC, Project 101132974 — OH SURVector.

PP 063

Utilising retrotransposon blood meal analysis (Rt-BMA) to identify *Ixodes ricinus* tick hosts and assess *Borrelia* infection in the United Kingdom

H. Broadhurst[1], *R. Birtles*[1], *K. August*[2], *S. Belmain*[2], *D. Bray*[2], *G. Massei*[3], *H. Marshall*[4], *S. Croft*[5]
H. Goethert[6], *S. Telford*[6]
[1]University of Salford, Salford, United Kingdom
[2]University of Greenwich, London, United Kingdom
[3]University of York, York, United Kingdom
[4]Forest Research, Edinburgh, United Kingdom
[5]Animal and Plant Health Agency, York, United Kingdom
[6]Cummings School of Veterinary Medicine, Boston, MA, United States

Tick-borne 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, hares, rabbits and birds) 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 to mitigate the risk of tick-borne diseases in the region.

PP 064

A resource-based habitat concept for ticks with a focus on forest as land systems

S. O. Vanwambeke[1], *L. Beautrix*[1], *M. Van Gestel*[2], *E. Matthysen*[2]
[1]UCLouvain, Earth & Life Institute, Louvain-la-Neuve, Belgium
[2]Univeristeit Antwerpen, Antwerp, Belgium

As complex socio-ecological systems, land systems result from sustained interactions and trade-offs made by diverse stakeholders. Ecosystem disservices related to health have so far scarcely been considered, despite the wealth of research associating land cover to zoonotic disease and land use to human exposure to infection. The land systems framework and its connection to health have great potential to further our understanding of tick-borne disease risk in forested landscapes. Many features of forests have been examined in the context of tick and tick-borne disease research, such as tree species, host abundance and diversity and undergrowth characteristics. Much less frequently, however, have these elements been examined in the perspective of forest management. Still, forest management is a dynamic field and in many regions of Europe where *Ixodes ricinus* is known to infest humans, and increasing attention is given to ecosystem resilience and biodiversity conservation. This focus on resilience and biodiversity may, however, be associated with heightened tick-borne disease risk. As forests are indeed under strong pressure from global environmental change, it appears timely to connect both fields of tick ecology and common forest management practices. In this research, we use the resource-based habitat framework to examine the connections between existing knowledge of tick habitat in forests, primarily of northwestern Europe, and forest management. We identify well-documented tick habitat features that are affected by forest managements (such as tree density, tree species and species mix, forest edge management) as well as forest management features that have drawn less attention in the field of tick research (e.g. volume of dead wood).

PP 065

Tick-borne pathogen prevalence and density of blacklegged ticks in four Pennsylvania counties

N. Chinnici[1], *S. Schwartz*[1], *M. Rowley*[1]
[1]East Stroudsburg University, Tick Research Laboratory of Pennsylvania, East Stroudsburg, PA, United States

Human pathogens vectored by ticks are a growing public health crisis, comprising over 75% of vector-borne disease cases in the United States. Ticks were collected using drag methods from 36 sites including schools, parks, and residential communities across four Pennsylvania (PA) counties in two geographical regions, northeast and northwest. A total of 16,160 adult and nymph blacklegged ticks were collected from 2022 through 2023. Blacklegged ticks were screened for five tickborne pathogens using qPCR assays to determine the prevalence of Powassan virus Lineage I and II, *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Babesia microti*, and *Anaplasma phagocytophilum* with variant distinction. Density of adult blacklegged ticks was higher in northwest PA compared to northeast PA, while there was no difference in nymph blacklegged tick densities between regions. All five tickborne pathogens were identified across the four PA counties in both adult and nymph blacklegged ticks.

PP 066

Ticks in the city versus in forests – mobile app shows risk of infection

K. Kybicová[1], E. Richtrová[1], J. Navrátil[1], V. Hönig[2], J. Kamiš[2], M. Kulma[3], P. Švec[4], P. Kukuliač[4]
Z. Vacek[5], J. Cukor[5], T. Václavík[6], J. Bartoška[7]
[1]National Institute of Public Health, National Reference Laboratory for Lyme Borreliosis, Prague, Czech Republic
[2]Biology Centre CAS, Laboratory of Arbovirology, České Budějovice, Czech Republic
[3]National Institute of Public Health, National Reference Laboratory for Vector Control, Prague, Czech Republic
[4]VSB – Technical University of Ostrava, Department of Geoinformatics, Ostrava, Czech Republic
[5]Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Prague, Czech Republic
[6]Palacky University Olomouc, Faculty of Science, Olomouc, Germany
[7]Czech University of Life Sciences Prague, Faculty of Economics and Management, Prague, Czech Republic

Lyme borreliosis (LB) and other tick-borne diseases are endemic in many countries in Europe including Czech Republic. The Czech Republic reports some of the highest LB incidences on the continent. The most widespread tick in this area is the common tick *Ixodes ricinus*, typically inhabiting forested areas with shrub and herbaceous layers. However, these ticks are increasingly found also in urban areas, where the public is less aware of the risks and less likely to protect themselves.

The aim of our ongoing work is to create a mobile application predicting tick activity and infection based on our extensive and regularly updated data on tick abundance and infection in selected urban and forest areas. Since 2014, we have been regularly collecting and testing ticks from 12 forest parks in Prague (4,845 ticks). We have added another 12 cities (6,620 ticks) and 30 smaller towns (4,032 ticks) starting from 2023. Finally, we have collected 60,416 ticks from 150 forest areas throughout the Czech Republic since 2021.

Preliminary results show that urban areas exhibit a higher tick abundance (15.7 ticks per 100 m^2) compared to forests (12.9 ticks per 100 m^2). Furthermore, ticks from urban environments have a higher infection rate with *Borrelia burgdorferi* s.l. (25.8%) and other pathogens, including *Borrelia miyamotoi* (2.4%), *Anaplasma phagocytophilum* (8.5%), *Neoehrlichia mikurensis* (2.0%), and *Rickettsia* spp. (7.0%). In contrast, ticks from forested areas show lower infection rates for *B. burgdorferi* s.l. (10.4%) and *A. phagocytophilum* (1.8%).

The mobile application “Klistapka” uses this data, time of the year and weather forecast to predict tick activity and infection risks, with emphasis on *B. burgdorferi* s.l., which is more prevalent in urban environments. Beyond providing real-time risk alerts, the application also educates users on preventive measures and health risks associated with tick bites.

The study was supported by the HADEA, EC, Project 101132974, OH SURVector

PP 067

Updated distribution of *Dermacentor* ticks in Austria

M. Ruivo[1], S. Purgatová[2], I. Hoxha[3], A. M. Schötta[4], I. Landsgesell[3], M. Markowicz[4]
G. G. Duscher[5], E. Kniha[3], M. Wijnveld[1]
[1]Medical University of Vienna, Institute for Hygiene and Applied Immunology, Vienna, Austria
[2]Slovak Academy of Sciences, Institute of Zoology, Bratislava, Slovakia
[3]Medical University of Vienna, Institute for Specific Prophylaxis and Tropical Medicine, Vienna, Austria
[4]AGES - Austrian Agency for Health and Food Safety, Institute for Medical Microbiology and Hygiene - Department for Vector-Borne Diseases, Vienna, Austria
[5]AGES - Austrian Agency for Health and Food Safety, Institute for Veterinary Disease Control, Mödling, Austria

Recent studies have shown that *Dermacentor* ticks are expanding to new areas in Europe. These ticks feed on ground-dwelling mammals, including humans, and are not known to feed on birds. As a result, their spread is limited to the range of these mammals and the obstacles they encounter, such as rivers, mountains, and other landmarks. In Austria, only limited studies have been conducted to investigate the distribution of *Dermacentor* ticks. Thus, we aimed to investigate the current occurrence, distribution, and associated pathogens of *Dermacentor* ticks.

Ticks were collected from the vegetation by hand in multiple locations in Vienna, Lower Austria, and Burgenland. All specimens were morphologically identified. A selection underwent molecular identification to confirm their taxonomic classification.

Subsequently, DNA was extracted from all collected ticks individually. PCR-reverse line blot analysis was used to identify the presence of pathogens (*Ehrlichia*, *Anaplasma*, *Babesia*, *Theileria*, *Rickettsia* and *Borrelia* spp.) in the obtained DNA extracts.

We have identified several novel locations where *Dermacentor* ticks are present. From these locations, we have collected a total of 856 specimens. Of all tested ticks, more than 80% were positive for at least one pathogen. The most common pathogens found in both *D. marginatus* and *D. reticulatus* were *Rickettsia* spp. Other pathogens detected were: *Borrelia burgdorferi* sensu lato, *Candidatus Neoehrlichia mikurensis* and *Theileria* (*Babesia*) *microti.*

The expansion of *Dermacentor ticks* to new geographical areas has led to an increased incidence of tick-borne pathogens in regions previously believed to be free of these ticks. *Dermacentor* ticks are known for their prolonged activity during winter months. This means that ticks are always active in Austria, not only during the spring, summer and autumn seasons.

This study will help to better understand the ecology and epidemiology of *Dermacentor* ticks and their associated pathogens.

PP 068

Ticks and the city: investigating ticks and tick-borne diseases in urban and peri-urban environments

M. Burdukiewicz[1,2], *J. Chilimoniuk*[1]
[1]Medical University of Białystok, Bialystok, Poland
[2]Vilnius University, Vilnius, Lithuania

Urban sprawl creates distinct habitats that allow ticks to come into contact with human populations, which increases the likelihood of transmitting tick-borne diseases. Several studies have investigated tick abundance, diversity, and pathogen prevalence in various cities, emphasizing the interactions among important urban factors (such as the fragmentation of green spaces), climate change, and tick ecology.

To understand the state of research and identify knowledge gaps in this field, we conducted a comprehensive meta-analysis of publications investigating ticks and TBDs in urban and peri-urban settings. Our study covered 10,805 peer-reviewed articles spanning diverse world cities. We evaluated trends in research focus, methodological approaches, and geographic coverage, identifying common patterns and regional differences. Key findings revealed that while tick diversity is often reduced in highly urbanized areas, peri-urban regions act as critical hotspots for both ticks and zoonotic pathogens.

PP 069

Predator-prey dynamics and tick-borne disease risk

N. De Pelsmaeker[1]
[1]Mammal Research Institute - Polish Academy of Sciences, Population Ecology, Białowieża, Poland

In Europe, red deer (*Cervus elaphus*) act as an important link in the life cycle of generalist ticks, and their abundance as hosts can influence both tick density as well as the prevalence of certain tick-borne pathogens. The presence of their main predator, grey wolves (*Canis lupus*), have been shown to alter the spatial behavior of deer, and the modified distribution can influence the availability and composition of tick hosts, affecting tick occurrence, density as well as pathogen prevalence, leading to potentially important implications for public health. In our ongoing project we investigate tick density and prevalence of *Borrelia burgdorferi s.l.* (the causal agent of Lyme disease), along a wolf-mediated gradient of deer presence, determined by camera trapping and historical data in the Białowieża Primeval Forest (Poland), to investigate whether predator-prey relations can affect disease risk. Additionally, wolf predation creates carcass sites which can decompose for several weeks, during which many different scavenger species visit these sites. As CO_2 is one of the primary cues used by ticks to locate a host, decomposition emanations may serve as an attractant, resulting in temporary tick hotspots. Such sites may constitute an enhanced contact point between ticks and hosts, and may provide an infection route for tick-borne pathogens to spread. We will test whether carcass volatiles can attract ticks by experimentally deploying red deer carcasses in the Bavarian Forest National Park (Germany) and performing a capture-mark-recapture experiment on ticks during the carcass decomposition process. Our project aims to fill the knowledge gap that exists on how large carnivores, via their impacts on ungulate prey species, can potentially modify tick abundance and disease prevalence on a landscape scale. This knowledge is highly relevant in the light of the ongoing recolonization of wolves that are increasingly dispersing and reestablishing in Europe.

PP 070

Unexplored ticks – studying lesser-known species as the key to understanding global pathogen vectors

D. Wężyk[1], M. Al-Sarraf[1], D. Dwużnik-Szarek[1], W. Romanek[1], L. Chitimia-Dobler[2], B. Boldbaatar[3]
K. Tołkacz[4], J. Behnke-Borowczyk[5], M. Ogrzewalska[6], A. Bajer[1]
[1]University of Warsaw, Eco-Epidemiology of Parasitic Diseases, Warsaw, Poland
[2]Bundeswehr Institute of Microbiology, Munich, Germany
[3]Mongolian University of Life Sciences, School of Veterinary Medicine, Ulaanbaatar, Mongolia
[4]Institute of Biochemistry and Biophysics PAS, Department of Antarctic Biology, Warsaw, Poland
[5]Poznań University of Life Sciences, Faculty of Forestry and Wood Technology, Poznań, Poland
[6]Oswaldo Cruz Foundation, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

Ticks that feed on a wide variety of hosts are vectors of the widest range of pathogens globally. There are at least two major gaps in tick research, including a focus on only three tick species commonly found in Europe and the US (*Ixodes ricinus, Ixodes scapularis, Dermacentor reticulatus*), and the use of ticks collected from hosts for such studies. The main aim of this project is to examine little-studied tick species from the genera *Hyalomma, Haemaphysalis, Amblyomma* and *Rhipicephalus* spp. for the presence of *Babesia*/*Theileria* and *Borrelia* spp.

Tick collections from the environment were conducted in various regions of the world, including Poland, Mongolia, Romania and Antarctica. In August 2023 and May 2024, two expeditions to Mongolia were performed where we managed to collect about 3,000 ticks from vegetation, including *Ixodes persulcatus, Dermacentor nuttalli* and *Hyalomma asiaticum*. In the initial phase of tick examination conducted in Mongolia, *Borrelia afzelii, Borrelia bavariensis* and *Babesia caballi*, *Babesia microti*, *Babesia venatorum*, *Babesia divergens* were identified. Concurrently, examinations of ticks originating from Romania unveiled the presence of *Babesia vogeli*, the causative agent of babesiosis in canines.

Acknowledgements: The study was funded by the National Science Centre, OPUS grant no. 2022/45/B/NZ7/01017 (AB).

PP 071

Ixodes laguri, a grassland and steppe tick that parasitizes critically endangered rodents

F. Rubel[1]
[1]University of Veterinary Medicine Vienna, Vienna, Austria

The range of *Ixodes laguri* Olenev, 1929 is only partially known. It is a nest-dwelling parasite of small mammals that mainly infest rodents of the families Cricetidae, Gliridae, Muridae and Sciuridae. There is no proven vectorial role for *I. laguri*, although it is suggested that it is a vector of *Francisella tularensis*. A first map depicting the entire range of *I. laguri* based on georeferenced locations and a list of main hosts is presented. For this purpose, a digital data set of 149 georeferenced locations from 16 countries was compiled. Particular attention is paid to the description of the westernmost record of *I. laguri* in the city of Vienna, Austria. There, *I. laguri* is specifically associated with its main hosts, the critically endangered European hamster (*Cricetus cricetus*) and the European ground squirrel (*Spermophilus citellus*). These two host species have also been mapped to estimate the potential distribution of *I. laguri* in the Vienna metropolitan region. The range of *I. laguri* extends between 16–108°E and 38–54°N, i.e. from Vienna in the east of Austria to Ulaanbaatar, the capital of Mongolia. In contrast to tick species that are expanding their range due to global warming, *I. laguri* has become increasingly rare throughout its range. However, *I. laguri* may be threatened not by climate change, but by anthropogenic influences on its hosts and their habitats, which are typically open grasslands and steppes. Rural habitats are threatened by intensification of agriculture and semi-urban habitats are increasingly being destroyed by urban development. Main hosts are hamsters (*C. cricetus, Nothocricetulus migratorius, Mesocricetus auratus, Mesocricetus newtoni, Mesocricetus raddei, M. brandti*) and ground squirrels (*S. citellus, S. pygmaeus*), but also gerbils (*M. unguiculatus, M. tristrami*) and marmots (*Marmota sibirica*). Less frequent *I. laguri* was reported from lemmings (*L. lagurus*), rodents such as mice, shrews and moles, and other small mammals.

PP 072

The understudied role of winter ticks (*Haemaphysalis inermis*) in pathogen transmission

É. Szabó[1,2], *D. A. Köves*[1,2], *M. Miklós*[1,2], *F. Kulin*[1,2], *M. Ruivo*[3], *M. Wijnveld*[3], *G. Földvári*[1,2]
[1]HUN-REN Centre for Ecological Research, Institute of Evolution, Budapest, Hungary
[2]National Laboratory for Health Security, Centre for Eco-Epidemiology, Budapest, Hungary
[3]Medical University of Vienna, Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Vienna, Australia

This study investigates the diversity and prevalence of pathogens associated with the winter tick (*Haemaphysalis inermis*), a species that has received limited attention despite its potential significance as a vector. Previous research suggests that *H. inermis* may transmit tick-borne encephalitis virus and other pathogens, such as *Rickettsia aeschlimannii*, *Rickettsia helvetica* (to humans), and *Babesia bigemina* (to livestock). However, its role in pathogen transmission and the full spectrum of pathogens it may harbour remain poorly understood.

Distributed widely across Eurasia, *H. inermis* is common in Central European forests during winter (November–February) and feeds on both humans and animals. A general perception of reduced tick activity in colder months may lead to underestimation of potential exposure risks, particularly as climate change contributes to milder winters and increased forest activity. These considerations underscore the need for accurate data on the vector potential of this species.

Over two years (October 2022–January 2023 and October–December 2023), 208 *H. inermis* ticks (123 females, 85 males) were collected and later analyzed using reverse line blot (RLB) to detect pathogens. Preliminary findings revealed that 48% of ticks tested positive for *Rickettsia* using a universal 16S rRNA probe, with *R. helvetica* being the predominant species. Some samples yielded signals suggestive of rare or unidentified *Rickettsia* species. Additionally, *Babesia* species were detected in 33% of ticks, while *Borrelia* and *Anaplasma* species were identified in approximately 10% of samples.

The diversity and abundance of pathogens associated with *H. inermis* exceeded initial expectations. Future research will focus on characterizing this species' vectorial role and the health risks posed by the pathogens it carries. Such insights are essential for informing public health strategies and policy decisions in the context of environmental and climatic changes.

PP 073

First report of *Ixodes inopinatus* from the Akrotiri wetland in Cyprus – Insights from tick surveillance and pathogen analysis

A. Saratsis[1], *K. Athanasiou*[2], *I. Angelidou*[2], *E. Apostolidi*[1], *R. E. Rollins*[3], *P. Ligda*[1], *E. Boukouvala*[1] *S. Sotiraki*[1], *L. Chitimia-Dobler*[4], *A. Martinou*[5]
[1]Hellenic Agricultural Organisation Dimitra, Veterinary Research Institute, Thermi, Greece
[2]Enalia Physis Environmental Research Centre, Nicosia, Cyprus
[3]Institute of Avian Research “Vogelwarte Helgoland”, Wilhelmshaven, Germany
[4]Bundeswehr Institute of Microbiology, Munich, Germany
[5]British Forces Cyprus, Joint Services Health Unit Cyprus, RAF Akrotiri, Cyprus

There are 19 tick species from five genera known in Cyprus, all found on animals. However, studies on questing ticks, their seasonality, and associated pathogens are lacking. Since April 2023 we have applied different surveillance methods (dragging, flagging, CO_2 trapping) to collect questing/hunting ticks, study their seasonality and the pathogens they carry. Collection occurred at the Akrotiri wetland protected area in Cyprus, an Important Bird Area (IBA) and RAMSAR site in the Eastern Mediterranean and Middle East region.

In March/April 2022, we recorded adult ticks morphologically belonging to the *Ixodes ricinus*/*inopinatus* complex for the first time in the planted, non-native *Eucalyptus* forest within the Akrotiri wetland. Since April 2023, biweekly monitoring of the tick population using the same methods has resulted in the collection of over 90 adult ticks at low densities, with activity occurring from November to April. Despite intensive sampling efforts, immature ticks were not collected. Phylogenetic analysis of the *TROSPA* gene showed that the ticks cluster with *I. inopinatus* reference sequences from Northern Africa. Molecular screening of a subset of ticks (n=76) for *Rickettsia* spp. and *Borrelia burgdorferi* s.l. by PCR-reverse line blot revealed *Rickettsia monacensis* (93.4% prevalence; 95% CI: 85.3-97.8%) and *Rickettsia helvetica* (1.3% prevalence; 95% CI: 0.03-7.1%) for the first time in Cyprus, but no *Borrelia* spp. infections. Molecular analysis for *Babesia*/*Theileria* spp. is still ongoing.

Our results raise concerns about risks to human and animal health. Given the lack of immatures in our collection, further research is needed to determine if the species has become established in the region and to assess its role in disease ecology.

PP 074

Using meta-analysis to estimate the incidence of the clinical manifestations of Lyme borreliosis in Denmark

<u>G. Brestrich</u>[1], A. Davidson[2], Y. Tan[3], J. Davis[4], F. J. Angulo[5], K. Halsby[6], J. Stark[7]
[1]Pfizer Pharma GmbH, Global Medical Affairs, Berlin, Germany
[2]Pfizer Inc., Global Medical Affairs, New York, NY, United States
[3]Pfizer, Evidence Generation Statistics, Cambridge, MA, United States
[4]Clarivate Analytics, Life Sciences and Healthcare, Boston, MA, Germany
[5]Pfizer Inc., Global Medical Affairs, Collegeville, PA, United States
[6]Pfizer Inc., Global Medical Affairs, Walton Oaks, United Kingdom
[7]Pfizer, Global Medical Affairs, Cambridge, MA, United States

Lyme borreliosis (LB) most commonly presents as erythema migrans (EM) but disseminated manifestations including arthritis (LA), neuroborreliosis (LNB) and other rare manifestations (OTH) can develop. Denmark conducts nationwide public surveillance for laboratory-confirmed LNB through reporting to the Staten Serum Institute (SSI); other clinical manifestations are not reported. The aim of this study is to estimate the incidence of all clinical manifestations of LB in Denmark in 2015-2023.

We derived estimates of all clinical manifestations for 2015-2023 in three steps. First, nationwide incidence of laboratory-confirmed LNB was obtained from SSI. Second, we estimated the ratios of other clinical manifestations to LNB reported in other European countries by performing a systematic literature review (SLR) of studies published between 01/01/2008 and 30/03/2024 and pooling the results using a random effects meta-analysis. Third, we applied the ratios to the reported LNB incidence from SSI to derive manifestation-specific incidence estimates.

The incidence of surveillance-reported LNB was 3.3/100,000 population per year (PPY) in Denmark in 2015-2023. The SLR identified 8 studies with pooled ratios of EM, LA and OTH manifestations to LNB of 42.81 (95% confidence interval [CI] 36.37-50.40), 0.85 (95% CI 0.71-1.02) and 0.76 (95% CI 0.42-1.36), respectively. The incidence of EM, LA, and OTH in 2015-2023 was 142.8/100,000 PPY, 2.8/100,000 PPY, and 2.5/100,000 PPY after applying these ratios to the mean LNB incidence. The overall LB incidence was 151.5/100,000 PPY in 2015-2023.

The burden of LB in Denmark is substantial. Estimation of all clinical manifestations is important to assess the health burden of LB and to inform healthcare providers and decision makers on the implementation of new treatments and preventions. Similar studies should be conducted across Europe with available data on LNB to better understand the full burden of LB.

PP 075

Incidence and manifestations of Lyme borreliosis in high-endemic sites in Germany – the Burden of Lyme Disease (BOLD) study

C. Kosch[1], K. Mehltretter[2], F. Johnson[3], J. Weimer[4], A. Loew-Baselli[5], K. Halsby[6], D. Yestekbayeva[6] J. Edwards[7], C. Malerczyk[8], C. von Eiff[8], F. J. Angulo[7], A. Pilz[5], Y. Tan[9], E. Begier[10], J. Stark[11]
[1]Praxis Dr. Kosch, Pirna, Germany
[2]Velocity Clinical Research, Leipzig, Germany
[3]Praxis Dr. Johnson, Rodgau, Germany
[4]Praxis Dr. Weimer, Reinfeld, Germany
[5]Pfizer, Global Vaccines and Anti-infectives Medical Affairs, Vienna, Austria
[6]Pfizer, Global Vaccines and Anti-infectives Medical Affairs, Walton Oaks, United Kingdom
[7]Pfizer Inc., Global Vaccines and Anti-infectives Medical Affairs, Collegeville, PA, United States
[8]Pfizer Pharma GmbH, Medical and Scientific Affairs, Berlin, Germany
[9]Pfizer, Evidence Generation Statistics, Pfizer Research and Development,, Cambridge, MA, United States
[10]Pfizer, Global Vaccines and Anti-infectives Medical Affairs, Dublin, Ireland
[11]Pfizer, Global Vaccines and Anti-infectives Medical Affairs, Cambridge, MA, United States

Introduction: Lyme borreliosis (LB) is the most frequent tick-borne disease in Europe, however disease incidence and clinical manifestations vary within and between countries. The BOLD study investigated LB incidence and clinical manifestations at selected sites in endemic regions of 6 European countries.

Methods: Five German sites were selected in Hesse, Schleswig-Holstein and Saxony. Active surveillance for medically attended suspect LB cases began in May 2021 and continued through 2022. Suspect LB cases were evaluated to determine if the patient met the EUCALB-derived clinical case definition. Research testing on enrolled subjects included a modified two-tiered ELISA (serum), culture and PCR (skin biopsy). Sites reported the number of patients served by the practice and the number of clinically diagnosed LB cases in 2019–2020, allowing annualization of LB case counts for 2021 and calculation of LB incidence for 2021 and 2022.

Results: Surveillance at the 5 trial sites identified 63 clinically diagnosed LB cases; 46 in 2021 and 17 in 2022. Overall, 41.3% of cases were male and the age-group with the most cases was 35–64 years (49.2%). The estimated incidence of clinically diagnosed LB cases per 100,000 population for 2021 and 2022 was 236.6 and 75.3, respectively. Of the 63 cases in Germany, 43 (68.3%) had erythema migrans (EM), 11 (17.5%) had Lyme arthritis (LA), 1 (1.6%) had Lyme neuroborreliosis (LNB), and 10 (15.9%) had other manifestations (e.g. systemic symptoms only). Two cases had >1 manifestation (EM/LA=1, LA/other=1). Among the 42 enrolled clinically diagnosed LB cases with samples available, 27 (64.3%) were laboratory confirmed, and the incidence of laboratory confirmed LB was 119.5 per 100,000 population per year.

Discussion: There was a high incidence of LB at the BOLD sites in Germany, supporting the need for public health interventions. Understanding the distribution of LB manifestations is important to assess the LB disease burden.

PP 076

Beware of hitchhiking ticks? Clarifying the variable roles of bird species in tick movement along migratory routes

L. Burnus[1], *J. Wynn*[1], *M. Liedvogel*[1], *R. E. Rollins*[1]
[1]Institute of Avian Research "Vogelwarte Helgoland", Wilhelmshaven, Germany

Ticks are blood-feeding parasites which act as major vectors for various pathogenic microorganisms affecting both animal and human health. Hard ticks are known to move passively (i.e. "hitchhike") on migratory birds as they transit between breeding and non-breeding grounds. This potentially leads to exchange and establishment of non-endemic tick species in novel environments. However, it is not yet clear if all migratory bird taxa play an equal role in movement of specific tick species, especially outside of medically important tick taxa. To clarify these interactions, we performed a systematic literature review regarding primary data of ticks moving on migratory birds within the African–Western Palearctic flyways. In total, 35 studies were found which showed 123 bird species from 37 families and 12 orders connected to potential movement of 30 tick species representing six genera (*Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus*). Most tick species did not show high abundance for any bird species, or, if they did, only on very few. Only *Ixodes ricinus* and *Hyalomma marginatum* were estimated to be carried at above average burdens by multiple bird species. This could indicate an increased likelihood of these species to be moved during migration. Specific tick species or whole genera were only found in certain migratory seasons as expected based on their geographic distributions. Even so, species found in both migratory seasons did not differ in their estimated abundances on birds between seasons. This result could suggest that tick abundance on migrating birds is not always a direct result of geographic distribution and may suggest an understudied importance of stopover sites towards potential tick introduction or turnover. Taken together, the results presented here provide guiding information for future analyses integrating individual level variation into the current understanding of tick movement with migratory birds.

PP 077

Borrelia burgdorferi s.l. in ticks from Lyme neuroborreliosis-risk areas in Denmark

N. Król[1,2,3]*, N. Skaarup Andersen*[2,4]*, L. Jung Kjær*[3]*, S. Løkkegaard Larsen*[2,4]*, F. Damgaard Nielsen*[4]
S. Skarphedinsson[2]*, R. Bødker*[3]*, M. Pfeffer*[1]
[1]University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany
[2]Odense University Hospital, Clinical Center for Emerging and Vector-Borne Infections, Odense, Denmark
[3]University of Copenhagen, Animal Welfare and Disease Control, Department of Veterinary and Animal Sciences, Frederiksberg, Denmark
[4]University of Southern Denmark, Research Unit of Clinical Microbiology, Odense, Denmark

Lyme neuroborreliosis (NB) is the most severe form of Lyme borreliosis and is primarily linked to *B. garinii* and *B. bavariensis*. However, it is unclear if all genotypes cause disease. The database for *Borrelia* multilocus sequence typing (MLST) shows a higher diversity of sequence types (STs) in ticks than in humans, suggesting that not all STs cause symptoms. This study explores *Borrelia* prevalence, genospecies, ST diversity, and NB risk in 3 area types of incidence: significant high, non-significant (medium risk), and significant low in Denmark.

In 2023, ticks were collected from the vegetation at 30 sites on Funen, Denmark, from high, medium, and low NB risk areas, based on reported incidence data from Odense University Hospital. Tick DNA was tested for *Borrelia* via qPCR with positive samples further analyzed using MLST targeting eight housekeeping genes. After next-generation sequencing, the results were compared to the *Borrelia* MLST database.

In total, 2007 *I. ricinus* ticks were collected, with most from high (42.8%) and medium (39.5%) risk areas. Nymphs (87.3%) were the dominant life stage. *Borrelia* DNA was found in 11.7% of ticks (n=234), with a slightly higher prevalence in medium risk area (13.9%) than in high and low risk areas (10.4% each). Infection rates were lowest in nymphs (10%), while for females (24.8%) and males (20.8%) were similar. So far, 24 samples out of 85 with Ct $\leq$ 37.5 have been sequenced revealing 7 genospecies, *B. afzelii, B. bavariensis, B. burgdorferi* s.s.*, B. garinii, B. spielmanii, B. valaisiana*, and *B. miyamotoi*. We observed high genotype diversity, 15 out of 24 samples had novel STs, 3 *B. afzelii* samples had known pathogenic profiles detected in patients from Germany and France, and 2 *B. afzelii* and *B. valaisiana* – had STs detected in ticks across Europe. Four samples could not be assigned.

Further analysis of this ongoing study will be presented at the conference. This work has been supported by the German Academic Exchange Service (DAAD).

PP 078

Prevalence of Borrelia burgdorferi sensu lato infections in Switzerland, 2022

R. Lienhard[1,2]*, R. Ackermann-Gäumann*[1,2]*, C. Niederhauser*[3]*, P. Gowland*[3]*, A. Croxatto*[1]
F. J. Angulo[4]*, A. Loew-Baselli*[5]*, G. Brestrich*[6]*, Y. Tan*[7]*, M. Covi*[8]*, A. Pilz*[5]*, J. Stark*[9]
[1]ADMED Microbiologie, Analyses et Diagnostics Médicaux, La Chaux-de-Fonds, Switzerland
[2]Swiss National Reference Center for Tick-transmitted Diseases, La Chaux-de-Fonds, Switzerland
[3]Swiss Red Cross, Interregional Blood Transfusion, Bern, Switzerland
[4]Pfizer Inc., Global Vaccines and Anti-Infectives Medical Affairs, Collegeville, PA, United States
[5]Pfizer Corporation Austria, Global Vaccines and Anti-Infectives Medical Affairs, Vienna, Austria
[6]Pfizer Pharma GmbH, Global Vaccines and Anti-Infectives Medical Affairs, Berlin, Germany
[7]Pfizer, Evidence Generation Staistics, Cambridge, MA, United States
[8]Pfizer AG, Vaccines and Medical Affairs, Zurich, Switzerland
[9]Pfizer, Global Vaccines and Anti-Infectives Medical Affairs, Cambridge, MA, United States

Introduction: Lyme borreliosis (LB), caused by *Borrelia burgdorferi* sensu lato (Bbsl), is the most common tick-borne disease in Europe. Seroprevalence studies provide estimates of the prevalence of people with anti-Bbsl antibodies (indicating current or past Bbsl infection) and are a useful tool for understanding the burden of LB and assessing the risk of acquiring Bbsl infection.

Methods: The study used 2,196 residual plasma samples from a SARS-CoV-2 seroprevalence study conducted from September to December 2022, collected from healthy 18–75 years old donors at seven blood donation service centers. At the Swiss LB National Reference Laboratory (NRL), samples were tested for anti-Bbsl IgG antibodies using the Liaison Borrelia IgG (DiaSorin), an anti-VlsE Assay as a screening test and the SeraSpot Anti-Borrelia-10 IgG (Seramun) as a confirmatory test to estimate seroprevalence. A model incorporating previously published data on the proportion of infections resulting in symptomatic disease and the duration of antibody detection was used to estimate the incidence of symptomatic Bbsl infection from the seroprevalence data.

Results: The prevalence of anti-Bbsl IgG antibodies was 6.1% (95% CI 5.1-7.1). Using estimates derived from the published literature that 37% of Bbsl-infected individuals are symptomatic, there were an estimated 14,603 (95% CI 11,491–16,039) symptomatic LB cases in Switzerland in 2022 if anti-Bbsl IgG antibodies can be detected for 10 years and 7,301 symptomatic LB cases (95% CI 6,104-8,498) if antibodies can be detected for 20 years. The estimated incidence of symptomatic infections was 226 (95% CI 178–248) and 113 (95% CI 94-131) per 100,000 population per year with 10-year and 20-year detection of anti-Bbsl IgG antibodies, respectively.

Conclusions: This study provides valuable insight into the burden of LB in Switzerland. The estimated incidence of symptomatic LB cases in 2022 underscores the importance of preventive measures against the disease.

PP 079

Diversity of ticks on trails in asturias (northwest Spain) – big differences depending on the study area

A. Espí Felgueroso[1], *A. Somoano García*[1], *A. L. D. Boone*[2], *M. J. Margolles Martins*[2]
[1]Regional Agri-Food Research and Development Service of the Principality of Asturias (SERIDA), Animal Health, Gijón, Spain
[2]Government of the Principality of Asturias, Department of Public Health, Oviedo, Spain

BACKGROUND: Located on the northern coast of Spain, Asturias offers travellers and residents awe-inspiring landscapes that can be enjoyed through its extensive network of hiking, biking and horseback riding trails. Nevertheless, due to its climatic and faunistic conditions, this region is also favourable for the presence of ticks, especially the *Ixodes ricinus* species, considered the main vector of Lyme borreliosis, a disease endemic to the area.

OBJECTIVES: Our aim is to assess the public health risk of tick bites on the busiest hiking trails of this autonomous region within the framework of a vector surveillance program for the Department of Health of Asturias.

METHODS: Between March 2023 and November 2024, a total of 1,477 questing ticks (96 larvae, 1,013 nymphs and 368 adults) were collected by drag sampling on 44 trails in Asturias. Collected ticks were identified based on morphological criteria and stored in 70 % ethanol for later analyses of pathogen communities.

RESULTS: Seven tick species of the genera *Ixodes*, *Haemaphysalis*, *Dermacentor* and *Rhipicephalus* were identified. *I. ricinus* accounted for 64.7% (238/368) and *D. reticulatus* for 22.5% (83/368) of adult ticks identified, whereas other species as *Haemaphysalis inermis*, *Dermacentor marginatus*, *Rhipicephalus bursa*, *H. concinna* and *H. punctata* accounted for 6.0%, 4.3%, 1.1%, 0.8% and 0.5% respectively.

CONCLUSIONS: As expected from previous studies in the region, *I. ricinus* was the most abundant species overall. However, on the trails closest to large population centers and during the coldest months of the year, *D. reticulatus* was the most abundant species. Although the analyses to detect pathogens in the collected ticks is still pending, in previous studies we found, a prevalence of 7.8% for *Rickettsia* spp. and 3.7% for *B. burgdorferi* s.l. in vegetation ticks. Therefore, we can consider that these pathogens are among the main risks for trail users.

PP 080

Burden of Lyme disease (BOLD) study – health-related quality of life among clinically diagnosed subjects with Lyme borreliosis by manifestation and compared with controls in six European countries

A. Mercadante[1], H. Yu[2], K. Halsby[3], A. Loew-Baselli[4], Y. Tan[5], F. J. Angulo[6], E. Begier[7], M. Dzingina[8]
J. S. Berglund[9], V. Cibik[10], K. Mehltretter[11], A. Moniuszko-Malinowska[12], L. Dybova[13], F. Strle[14]
D. Zakova[15], J. Stark[16]
[1]Pfizer Inc., HTA Value and Evidence, New York, NY, United States
[2]Pfizer Inc., HTA Value and Evidence, Collegeville, PA, United States
[3]Pfizer, Global Vaccines and Anti-infectives Medical Affairs, Surrey, United Kingdom
[4]Pfizer, Global Vaccines and Anti-infectives Medical Affairs, Vienna, Austria
[5]Pfizer, Evidence Generation Statistics,, Cambridge, MA, United States
[6]Pfizer, Global Vaccines and Anti-infectives Medical Affairs, Collegeville, PA, United States
[7]Pfizer, Global Vaccines and Anti-infectives Medical Affairs, Dublin, Ireland
[8]Pfizer, Global Access and Value, Primary Care, Walton Oaks, United Kingdom
[9]Blekinge Institute of Technology, Department of Health, Karlskrona, Sweden
[10]MUDr. Viliam Cibik, PhD, s.r.o., Pruske, Slovakia
[11]Velocity Clinical Research, Leipzig, Germany
[12]Medical University of Białystok, Bialystok, Poland
[13]Doktor Brno s.r.o., Brno, Czech Republic
[14]University Medical Centre Ljubljana, Department of Infectious Diseases, Ljubljana, Slovenia
[15]MUDr. Zakova, s.r.o, Ambulancia vnutorneho lekarstva, Trencin, Slovakia
[16]Pfizer, Global Vaccines and Anti-infectives Medical Affairs, Cambridge, MA, United States

Background: Lyme borreliosis (LB) is the most common tick-borne disease in Europe with over 129,000 surveillance-reported cases per year. Health related quality of life (HRQoL) outcomes from patient reported outcome (PRO) measures illustrate patients' perception of their disease and its impacts on their lives. This study investigated HRQoL, severity of fatigue, pain and cognitive function in six European countries (Czechia, Germany, Poland, Slovakia, Slovenia, and Sweden).
Methods: Clinically diagnosed LB cases among 12 primary care practices in 2021 and 2022 were recruited at diagnosis and followed for 10 months across three visits (V1-V3). One age-matched control per case was recruited from the practice panels of the LB cases. HRQoL, pain, fatigue, and cognitive function were measured using PROs: SF-36, SF-MPQ, FSS, and CFQ. Average domain or total scores were compared between LB manifestation subgroups and with matched controls.
Results:Of the 315 clinically diagnosed cases with LB, 210 and 105 had erythema migrans (EM) and disseminated disease (DD) manifestations (e.g., neuroborreliosis, arthritis, carditis, etc.), respectively. For all LB cases, there was an improvement in the scores of fatigue and bodily pain from V1 to V3. SF-36 Bodily Pain Subscale, SF-36 Physical Function Subscale, and SF-MPQ scores were significantly higher in cases with DD than EM from V2 to V3; FSS scores were significantly higher in V1, V2 and V3. Fatigue and bodily pain scores were significantly worse in DD cases than controls at V1 and V2.
Conclusion: LB cases with DD reported significantly increased symptoms severity and reduced HRQoL than those with EM. Cases with DD also documented worse HRQoL scores at most time points compared to controls. A vaccine would prevent the negative impact of LB on patients' quality of life, particularly those with disseminated manifestations.

PP 081

Ticks and tick-borne pathogens in urban areas in Lithuania

J. Radzijevskaja[1], *J. Snegiriovaitė*[1], *I. Lipatova*[1], *M. Razgūnaitė*[1], *A. Paulauskas*[1]
[1]Vytautas Magnus University, Research Institute of Natural and Technological Sciences, Kaunas, Lithuania

Recent studies in Europe highlight the growing risk of tick-borne diseases (TBDs) due to urbanization and ecological changes. Lithuania is a highly endemic area for tick-borne encephalitis and Lyme borreliosis (LB), with *Ixodes ricinus* known to carry various tick-borne pathogens (TBPs). In addition to the agents of these notifiable diseases, various other TBPs, such as *Anaplasma phagocytophilum*, *Rickettsia helvetica*, and several *Babesia* species, have been detected in questing *I. ricinus* in their natural habitats. However, the prevalence of these pathogens in urban and suburban areas remains largely unexplored. This study investigated the distribution of *I. ricinus* ticks and their infection rates with *Borrelia* spp., *A. phagocytophilum*, and *Neoehrlichia mikurensis* in urban green spaces in Lithuania. Ticks were collected from 34 urban and 17 peri-urban locations, including public parks, city forests, botanical gardens, and regional parks, during 2021–2023. Pathogens were screened using triplex real-time PCR, followed by PCR and sequencing for positive samples. A total of 3,599 ticks were collected from 83% of the surveyed sites, which included all peri-urban and 25 urban sites, with the highest abundance found in forest parks. *Borrelia* DNA was detected in 24.06% of the ticks, while *A. phagocytophilum* and *N. mikurensis* were found in 4.85% and 4.88% of the ticks, respectively. Six *Borrelia* species were identified: *B. afzelii*, *B. garinii*, *B. burgdorferi* s.s., *B. valaisiana*, *B. lusitaniae*, and *B. miyamotoi*. The prevalence of TBPs varied among urban and peri-urban locations and was higher in adult ticks compared to nymphs. This study emphasizes the importance of monitoring tick populations in urban green spaces, which pose a significant risk for TBD transmission, particularly LB, anaplasmosis, and neoehrlichiosis. Notably, *N. mikurensis* and *B. lusitaniae* were detected in Lithuania for the first time.

Funding: Research Council of Lithuania (Grant No. S-MIP-23/19).

PP 082

Molecular detection of *Borrelia* spp. in red squirrels (*Sciurus vulgaris*) and their ticks

U. Medikaitė[1], *I. Lipatova*[1], *J. Snegiriovaitė*[1], *J. Radzijevskaja*[1], *A. Paulauskas*[1]
[1]Vytautas Magnus University, Department of Biology, Kaunas, Lithuania

Lyme disease, caused by *Borrelia burgdorferi* sensu lato, represents the most prevalent vector-borne disease in North America and Europe, with high incidences reported in Germany, Slovenia, and Lithuania. The expanding distribution of ticks and their hosts has contributed to the increasing number of cases over recent decades. Among potential hosts, *Sciurus vulgaris* (red squirrel) is of particular interest due to its widespread distribution in both natural and urban environments, where it frequently comes into contact with ticks, primary vectors of Lyme disease. The red squirrel may act as a reservoir host for *B. burgdorferi* s.l. and increase human exposure to tick-borne pathogens. The study aimed to detect the presence of *Borrelia* species in red squirrels and the ticks collected from them in various parks in Kaunas city. A total of 45 red squirrel samples were analyzed using real-time PCR with primers specific to *Borrelia*. Samples that tested positive for *Borrelia* DNA were further examined with a PCR amplifying fragments of *ospA* and *IGS* regions, and positive PCR products were chosen for DNA sequencing. The study found *Borrelia* species in 20.0% of the squirrels by *IGS* and 17.7% by *ospA* gene. From all 117 collected ticks, belonging to the species *Ixodes ricinus*, *Borrelia* was detected in 14.5% by *IGS* and 5.9% by *ospA* gene. We identified three *Borrelia* species causing Lyme disease: *B. afzelii*, *B. garinii* and *B. burgdorferi* sensu stricto. These results highlight the potential role of red squirrels in spreading Lyme disease pathogens within urban environments and underline the need for continued monitoring of wildlife in tick-endemic regions.

ACKNOWLEDGMENTS. Part of this research was funded by European Social Fund under grant agreement P-ST-24-154 with the Research Council of Lithuania.

PP 083

The raccoon (*Procyon lotor*) as an emerging neozoon and potential reservoir for tick-borne pathogens in Germany

L. M. I. Maas[1], *N. Król*[1], *Z. Rentería-Solís*[2], *T. Langner*[3], *N. P. Reinhardt*[4], *M. Pfeffer*[1], *S. Birka*[3] *P. S. Sebastian*[5], *A. Obiegala*[1]
[1]University of Leipzig, Institut für Tierhygiene und Öffentliches Veterinärwesen, Leipzig, Germany
[2]University of Leipzig, Institut für Parasitologie, Leipzig, Germany
[3]University of Leipzig, Institut für Lebensmittelhygiene, Leipzig, Germany
[4]Environment and Consumer Protection North Rhine-Westphalia, Wildlife Research Institute, Bonn, Germany
[5]IdICaL; CONICET-INTA, Dairy Chain Research Institute, Rafaela, Argentina

The raccoon (*Procyon lotor*) is a medium-sized omnivore belonging to the family of small bears (Procyonidae) that originally belongs to Central and Northern America. Nowadays, it has colonized different parts of the world due to deliberate or accidental releases and is listed as invasive neozoon in Germany. The recent rise in population densities is likely to increase the risk of pathogen transmission to humans, wildlife and domestic animals. Many zoonotic pathogens are found in raccoons worldwide, but there is a lack of epidemiological data for most of Germany's raccoon populations concerning tick-borne pathogens.

Tissue samples of 485 free-ranging raccoons obtained as hunting bag in ten federal states of Germany between 2017 and 2021 were examined for the presence of five vector-borne pathogens (*Rickettsia* spp., *Borrelia burgdorferi* sensu lato (s.l.), *Bartonella* spp., *Babesia* spp., *Neoehrlichia mikurensis*) using molecular methods.

Borrelia burgdorferi s.l. was detected in 21 (6.3%) raccoons, identified as *Borrelia afzelii* and *Borrelia burgdorferi* s.s. Multilocus Sequence Typing (MLST) of the *Borrelia burgdorferi* s.l. positive samples was also conducted, to our knowledge for the first time in samples deriving from raccoons. *Rickettsia* spp. were found in 26 (7.8%) individuals, identified as *Rickettsia helvetica* ($n=5$), *Rickettsia felis* ($n=2$) and *Rickettsia conorii subsp. raoultii* ($n=1$). *Bartonella* spp. were confirmed in 3 (0.6%) raccoons. *Neoehrlichia mikurensis* and *Babesia* spp. were not detected.
Future studies should monitor these invasive omnivore populations. However, raccoons may act as reservoir for pathogens, especially for *B. burgdorferi* s.l. and *Rickettsia* spp., between humans, domestic animals, zoo animals, and wildlife, with a risk of infection due to their invasive behaviour and synanthropic habitat.

PP 084

Nationwide survey of *Borrelia* prevalence and species distribution in *Ixodes ricinus* ticks in Germany

J. R. Raasch[1], *A. Springer*[1], *M. Pfeffer*[2], *U. Mackenstedt*[3], *O. Kahl*[4], *C. Strube*[1]
[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hanover, Germany
[2]University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany
[3]University of Hohenheim, Institute of Biology, Department of Parasitology, Stuttgart, Germany
[4]tick-radar GmbH, Berlin, Germany

As a vector for various pathogens, in particular those causing Lyme borreliosis, *Ixodes ricinus* is the most important tick species in large parts of Europe and also Germany. The aims of this study are to obtain a representative Germany-wide estimate of *Borrelia* prevalence in questing *I. ricinus* nymphs, to identify regional differences, and to determine the *Borrelia* species distribution. The ticks were collected at various locations throughout Germany during 2018-2020. If available, 300 nymphs per location will be examined by quantitative real-time PCR, resulting in an approximate sample size of 12,000 specimens. First results for 15 locations in the regions of Hanover, Emsland, Bremen, Uelzen and Kassel for the years 2018 and 2019 have already been published. In this north-western German region, the *Borrelia* prevalence in questing nymphs was 28.6% (300/1,050) [1]. Among 48 further locations analysed so far for 2018, an overall prevalence of 32.8% (1,105/3,373) was determined. The preliminary data indicate possibly higher prevalences in northern Germany, e.g. in Stralsund with 31.7% (95/300) and Schwerin with 35.0% (105/300), compared to southern regions, e.g. Erlangen with 22.4% (35/156) and Schrobenhausen near Munich with 20.3% (36/177). The *Borrelia* prevalence estimation in questing *I. ricinus* nymphs is ongoing, and additionally first results of the *Borrelia* species differentiation, including the relapsing fever spirochaete *Borrelia miyamotoi*, will be presented.

Reference:

1. Knoll, S., Springer, A., Hauck, D., Schunack, B., Pachnicke, S., Fingerle, V., Strube, C. (2021). Distribution of *Borrelia burgdorferi* s.l. and *Borrelia miyamotoi* in *Ixodes* tick populations in Northern Germany, co-infections with Rickettsiales and assessment of potential influencing factors. Med Vet Entomol 35: 595-606

PP 085

Vector-free transmission of Lyme disease spirochetes

N. Rudenko[1], M. Golovchenko[1]
[1]Biology Centre CAS, Ceske Budejovice, Czech Republic

Lyme borreliosis (LB) is one of the most frequently recorded tick-borne diseases in the Northern Hemisphere, caused by selected species of spirochetes from the *Borrelia burgdorferi* sensu lato complex, transmitted from infected tick to the vertebrate host, including humans, during the tick bite. Transmission of tick-borne pathogens is clearly via ticks. This primary mode of bacterial transmission was established when the connection between the LB, ticks and spirochetes was first discovered. But are there other routes of transmission? The rapid increasing of LB cases and its spreading might be due to several factors: changes in the distributional range of tick vectors; dispersal of infected ticks due to host animal migration; recent urbanization and an increasing overlap of human, wildlife, ticks and *Borrelia* habitats; possible involvement of other bloodsucking arthropod vectors or possibly, other routes of transmission of the causative agent. Even a minor component of the pathogen transmission managed without involvement of its vector is important medically and economically already today, and will become even more so in the future. LB shares many features with the other spirochetal diseases for which the existence of vector-free transmission is well documented: 1) skin or mucous membrane as an entry point; 2) spirochetemia early in the course of disease, with wide dissemination through tissue and body fluid with intervening latent periods, leading to the risk of transmission through blood transfusions; 3) survival of viable spirochetes in human genital secretions; and 4) tropism for skin, neurologic or cardiovascular tissues. Given the knowledge and our understanding of vector-free transmission for other spirochete species, non-vectorial transmission of LB spirochetes is biologically highly plausible. Even though, it is almost impossible to find studies that address non-vectorial transmission of LB spirochetes, there are no studies that clearly deny its existence.

PP 086

Concurrent infection of the human brain with multiple species of Lyme disease spirochetes

N. Rudenko[1], *M. Golovchenko*[1], *J. Opelka*[1], *M. Vancova*[1], *H. Sehadová*[1], *M. Raska*[2], *M. Krupka*[3]
[1]Biology Centre CAS, Ceske Budejovice, Czech Republic
[2]University Hospital Olomouc, Olomouc, Czech Republic
[3]Palacky University Olomouc, Olomouc, Czech Republic

Lyme disease (LD) spirochetes are well known to be able to disseminate into the tissues of infected hosts, including humans. The diverse strategies used by spirochetes to avoid the host immune system and persist in the host include active immune suppression, induction of immune tolerance, phase and antigenic variation, intracellular seclusion, changing of morphological and physiological state in varying environment, formation of biofilms and persistent forms, and, importantly, incursion into immune privileged sites such as the brain. Invasion of immune privileged sites allows the spirochetes not only escape from the host immune system but also reduce the efficacy of antibiotic therapy. Here we present a case of the detection of spirochetal DNA in multiple loci of a LD patient's post-mortem brain. The presence of co-infection with *Borrelia burgdorferi* sensu stricto and *Borrelia garinii* in this LD patient's brain was confirmed by PCR. The presence of atypical spirochete morphology was confirmed by immunohistochemistry of the brain samples and, previously, also in tissues of infected with *Borrelia* experimental mice, that were used as a control in the adjustment of the protocol for analysis of human brain samples. Even though both spirochete species were simultaneously present in the human brain tissue, the brain regions where the two species were detected were different and non-overlapping.

PP 087

Health claims data reveal high incidence of tick-borne encephalitis in Germany

G. Levy[1]*, I. Karkossa*[2]*, J. Schiffner-Rohe*[1]*, C. von Eiff*[1]*, G. Dobler*[3]*, C. Malerczyk*[1]
[1]Pfizer Pharma GmbH, Berlin, Germany
[2]Gesundheitsforen Leipzig GmbH, Leipzig, Germany
[3]Bundeswehr Institute of Microbiology, Munich, Germany

Introduction: Mandatory reporting for tick-borne encephalitis (TBE) in Germany was introduced in 2001. Since then, the Robert-Koch Institute (RKI) reported between 195-705 annual cases fulfilling the case definition, which prerequires a confirmatory laboratory test. The current study aims to examine the incidence of TBE in claims data and to compare it with the cases reported by the RKI.

Methods: Claims data from the German Analysis Data Bank for Evaluation and Healthcare Research (DADB), comprising up to 3 million statutory health insured (SHI) patients annually between 2013-2022, were analyzed in a retrospective cohort study. The data are representative for Germany regarding regional distribution, age and sex. Incident TBE patients were identified based on ICD-10 A84 diagnoses, coded as a confirmed outpatient or a main/secondary inpatient diagnosis, for whom no TBE diagnosis was coded in the previous year. Confounding variables such as diagnostic tests performed, concurring diagnoses, treatment setting, hospitalization and mortality were also examined. Analyses were stratified by age, sex and region.

Results: First results revealed 2,572 incident TBE patients between 2014 and 2022 in the DADB, of those 97% were first diagnosed in the outpatient setting. Extrapolated to the SHI population, this corresponds to 71,157 (CI: 66,971-73,434) cases and constitutes about 17-fold the 4,054 cases reported by the RKI for the same timeframe. A specific laboratory test was conducted in only 2% of the DADB cases.

Conclusion: While many more TBE cases were detected in claims data than reported by the RKI, reasons for this discrepancy are unclear and must be determined. Low diagnostic test rates identified in the DADB may contribute to this finding, yet additional aspects such as underreporting and underdiagnosis may have also played a role. Further research is required to gain more insights into clinical practice, coding and laboratory diagnostics.

This research was sponsored by Pfizer Pharma GmbH

PP 088

An update on the estimate of Lyme disease incidence in England

J. Olver[1]*, C. Petridou*[1]*, J. Edney*[2]*, R. Vivancos*[3,4,5]*, A. Semper*[1]
[1]UK Health Security Agency, Rare and Imported Pathogens Laboratory, Salisbury, United Kingdom
[2]UK Health Security Agency, Field Service South East and London, London, United Kingdom
[3]UK Health Security Agency, Field Service North West, Liverpool, United Kingdom
[4]University of Liverpool, National Institute for Health Research, Health Protection Research Unit in Gastrointestinal Infections, Liverpool, United Kingdom
[5]University of Warwick, Warwick Medical School, Coventry, United Kingdom

Introduction

Lyme disease (LD) is not notifiable in the UK. This means that official incidence figures for England are based on the number of cases with a laboratory-confirmed diagnosis at the UK Health Security Agency (UKHSA) reference laboratory.

A previous study that analysed primary care data in The Health Improvement Network (THIN) from 1998 to 2016 found that for every laboratory-confirmed case there are 2.35 cases identified within a primary care setting. This number can be used as a multiplication factor to estimate the incidence of acute clinical cases of LD from the official incidence figures. It is suspected that this multiplication factor may now be out of date, partly due to the introduction of national guidance in 2018, which recommend that patients presenting with a pathognomonic erythema migrans rash should be diagnosed without laboratory-confirmation.This study aimed to provide an updated estimate of LD incidence in England.

Methods

We analysed time series models in R of both the number of acute cases of LD diagnosed within primary care and those diagnosed at the national reference laboratory at UKHSA between 1st January 2013 and 31st December 2023. The primary care data was derived from the Clinical Practice Research Datalink (CPRD), which collects data from primary care practices in the UK. Data was pulled using Read codes and prescribing data relevant to Lyme disease.

Results

Initial data analysis found no evidence to suggest a sudden change in the number of monthly laboratory-confirmed acute LD cases. However, there was a trend for a gradual increase in the number of these cases from 2013 to 2023. Similarly, there was a trend for a gradual increase in the number of acute LD cases diagnosed within primary care, with a potential sudden increase in May 2016. Future analysis aims to determine whether this will significantly affect the multiplication factor.

PP 089

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

G. Plahe[1], *S. L. Gandy*[2], *J. L. Hall*[2], *D. Johnson*[3], *L. Gilbert*[2], *R. Birtles*[1]
[1]University of Salford, School of science, engineering and environment, Salford, United Kingdom
[2]University of Glasgow, Glasgow, United Kingdom
[3]University of Manchester, Manchester, United Kingdom

Acquisition of Lyme disease, caused by the tick-borne pathogen *Borrelia burgdorferi s.l.* is generally thought to occur in rural settings, such as woodlands and rough pasture. However, increasingly, the Lyme disease hazard of urban greenspaces is being recognised, particularly as these areas have higher footfall than their rural counterparts[1].

We hypothesize that tick populations in urban greenspaces are derived from those in the rural hinterland surrounding them and that the tick and Lyme disease hazard of an urban greenspace is influenced by the surrounding hinterland[2]. We also hypothesise that *B. burgdorferi s.l.* ecology in urban greenspaces is less likely to mimic the surrounding hinterland as local communities of tick hosts and *B. burgdorferi s.l.* reservoirs will differ. To test these hypotheses, questing ticks, and associated field data, were collected from urban and rural green spaces in 20 towns and cities across the UK in 2022, 2023, and 2024. DNA was extracted from ticks and the presence and identity of the infecting *Borrelia* genospecies was established. Analysis found that the densities of nymphs at hinterland sites significantly correlated with those at urban sites[2]. However, the Lyme disease hazard (density of infected nymphs) was significantly lower at urban sites than rural sites and the relative abundance of *Borrelia* genospecies differed significantly. These results support our hypotheses and provide key information that improves our understanding of Lyme disease hazard in urban greenspaces.

1. Hansford, K. M., Wheeler, B. W., Tschirren, B., & Medlock, J. M. (2022). Questing *Ixodes ricinus* ticks and *Borrelia* spp. in urban green space across Europe: A review. Zoonoses Public Health, 69(3), 153-166. https://doi.org/10.1111/zph.12913
2. Gandy, S. L., Hall, J. L., Plahe, G., Watkinson, K., Johnson, D., Birtles, R, J. & Gilbert, L. (2024). The health hazards of ticks and Lyme disease in towns depend on the wider hinterland context. [unpublished manuscript].

PP 090

First findings of *Babesia* spp. and *Rickettsia* spp. in bats (Chiroptera) in Slovakia

Ľ. Korytár[1], A. Ondrejková[1], M. Prokeš[1], J. Lipinský[1], F. Hake[1], Y. M. Didyk[2,3], S. Purgatová[2]
B. Mangová[2], M. Ruivo[4], M. Wijnveld[4], M. Kazimírová[2]
[1]University of Veterinary Medicine and Pharmacy in Košice, Košíce, Slovakia
[2]Slovak Academy of Sciences, Institute of Zoology, Department of Medical Zoology, Bratislava, Slovakia
[3]Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, Ukraine
[4]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Vienna, Austria

Bats are reservoirs of zoonotic pathogens and parasites worldwide. Piroplasmida blood parasites and *Rickettsia* spp. infect bats and bat ticks, but data on their host range, vectors, and geographical distribution are limited. *Babesia vesperuginis* and *Rickettsia* spp. of the spotted fever group (SFG) were detected in bats and bat ticks in a few European countries, but there is no information on their associations with bats in Slovakia. This study was aimed at the investigation of piroplasmids and *Rickettsia* spp. in bats and their ticks in Slovakia. Bats were trapped by mist-nets (Ecotone, Poland) at several localities in the cadastre of Drienovec village (Slovak karst). Trapping and sampling of bats were performed in autumn 2022 based on permission No. 3051/2019-6.3 provided by the Ministry of Environment of the Slovak Republic. From 17 specimens of *Myotis myotis,* EDTA blood was collected. *Carios vespertilionis* ticks were collected from *Pipistrellus pipistrellus.* DNA was isolated from the blood and ticks. Blood was analysed for piroplasmid DNA by PCR and by reverse line blotting (RLB), both targeting the 18S rRNA gene, and for *Rickettsia* spp. by RLB targeting the 16S and 23S-5S IGS genes. Tick DNA was analysed for *Rickettsia* spp. by PCR targeting the sca4 gene. *Babesia* spp. were identified in the blood of seven bats (41.2%). Bidirectional sequencing of a part of the 18S rRNA gene confirmed the presence of *B. vesperuginis* and *B. microti*. *Rickettsia* spp. DNA was identified in the blood of five bats (29.4%), but the sequences need further analyses. SFG *Rickettsia* spp. were found in two ticks. These are the first confirmed infections of bats with *B. vesperuginis* and *Rickettsia* sp. and of bat ticks with SFG rickettsiae in Slovakia. Our study widens the knowledge on the geographic distribution of *B. vesperuginis* and on associations of bats and bat ticks with rickettsiae. Acknowledgements. This work was supported by the project of Action Austria-Slovakia SAIA 2023-10-15-001.

PP 091

Ectoparasites associated with rodents in different regions of Iran

N. Moradpoor Sheykhkanloo[1,2], A. Moshaverinia[2], H. Borji[2], A. Mahmoudi[3]
[1]Ustav biologie obratlovcu akademie ved cr, KONEŠIN/Studenec, Czech Republic
[2]School of Veterinary Medicine, Ferdowsi University of Mashhad, Pathobiology, Mashhad, Iran
[3]Urmia University, Faculty of Science, Biology, Urmia, Iran

Iran is a big country with different climate zones, providing ideal conditions for rodents and their parasites. Iran's climate zones can be classified into three broad types. The first zone is characterized by high humidity and wet habitats, occurring mainly along the southern margins of the Caspian Sea. Most of our sampling occurred in the second zone, which consists of relatively cold, mountain areas in eastern and western parts of the country. The third area includes the arid and crop and semi-arid dry areas. This survey aims to evaluate the distribution of rodent species in different regions of Iran and their relationship with ectoparasite infestation. Of the 200 rodent captures, 160 showed infestation by various arthropod parasites. These parasites were belonging to four orders and six families: Phthiraptera (Polypalcidae, Hoplopleuridae), Siphonaptera (Ctenophthalmidae), Metastigmata (Ixodidae, *Hyalomma*) and Mesostigmata (Laelapidae, Macronyssidae). The humid Caspian region had the highest values of ectoparasite infestation. Considering the importance of some of these ectoparasites as zoonotic disease vectors, prevention strategies should help local health authorities to avert public health risks.

PP 092

Temporal changes in tick-borne pathogens prevalence observed in questing *Ixodes ricinus* (Linnaeus, 1758) across different habitat types in north-eastern Italian Alps

G. Ferrari[1,2], *F. Rosso*[1], *V. Tagliapietra*[1,2], *T. Weil*[1], *G. Marini*[1], *F. Dagostin*[1], *D. Arnoldi*[1], *M. Girardi*[1]
A. Rizzoli[1,2]
[1]Fondazione Edmund Mach, San Michele all'Adige, Italy
[2]National Biodiversity Future Center, Palermo, Italy

Changes in land use, climate, and host community lead to increased complexity in eco-epidemiological relationships and zoonotic emergence. This study investigates the changes in the prevalence of *Ixodes ricinus*-transmitted pathogens in questing ticks over a 10-year interval in natural and agricultural habitats of the Autonomous Province of Trento (North-eastern Alps).

We investigated three study sites classified as natural and agricultural by collecting questing ticks in 2011-2013, 2020 by tick-dragging along 100 m transects. The collected ticks were morphologically identified, while bacteria and protozoa were detected using PCR-based methods combined with sequencing. We investigated the effect of habitat type, tick stage and year on the proportion of infected ticks by using Generalized Linear Models (GLMs) and Two Proportion Z-test.

In total 2652 ticks belonging to *I. ricinus* were collected and analysed. We found an overall prevalence of 27.1% in the study area, referred to *Borrelia* spp. (21.1%), *Rickettsia* spp. (8.4%) *Anaplasma phagocytophilum* (1.6%) and *Babesia* spp. (0.4%). We reported the circulation of 11 different zoonotic pathogen species, with varying infection rates across different years and habitats. In 2020, *A. phagocytophilum*, associated with agricultural habitats decreased, while *Babesia* spp. was found in all habitats. Among *Borrelia burgdorferi* s.l. complex, only *B. burgdorferi* s.s. and *B. lusitaniae* showed significant relationships in natural habitats, increasing in 2020. *Rickettsia* spp. did not show any significant habitat preference. Co-infections were identified in 8% of positive-tested ticks with different spatio-temporal associations, primarily in natural settings.

Our results provide new evidences that the risk of infection with tick-borne pathogens in the Alpine region varies over time and in different environments, broadening the current information on coinfection rates and circulation of zoonotic pathogens, previously not reported in this area.

PP 093

Environmental influences on tickborne pathogen prevalence and *Peromyscus leucopus* population size in Pennsylvania, USA

M. Rowley[1], *S. Schwartz*[1], *G. Davies*[1], *M. Williams*[1], *P. Langlais*[1], *G. Oram*[1], *N. Chinnici*[1]
[1]East Stroudsburg University, Pennsylvania Tick Research Lab, East Stroudsburg, PA, United States

Tickborne pathogens (TBPs) present a significant public health concern and require integrative approaches to monitor and mitigate their effects on humans. To better understand the ecological context of TBPs, we must consider the reservoir species that facilitate their spread among ticks. In this study, we estimated the population size and recruitment/loss rates of *Peromyscus leucopus*, a known reservoir of TBPs, across 36 sites in Northeastern and Northwestern Pennsylvania (with n = 1,949 and n = 1,077 capture events, respectively). We also surveyed abiotic and biotic environmental variables, including microclimate and vegetation structure, to evaluate their influence on *P. leucopus* population size. These efforts, when paired with assessments of TBP infection rates, provide a basis of comparison for determining the effectiveness of future mitigation treatments.

PP 094

Knowledge, attitudes, and practices regarding tick bites and tick-borne diseases – insights from high-risk groups at an *Ixodes ricinus* hotspot in Greece

A. Saratsis[1], *E. Kondilis*[2], *A. Benos*[2]
[1]Hellenic Agricultural Organisation Dimitra, Veterinary Research Institute, Thermi, Greece
[2]Aristotle University of Thessaloniki, Laboratory of Primary Health Care, Thessaloniki, Greece

Despite improvements in diagnosis and treatment of tick-borne diseases (TBDs), absence of effective tick population control measures in nature lead to the conclusion that primary prevention still constitutes the cornerstone of TBD incidence reduction. The objective of this study was to determine the knowledge, attitudes and practices regarding ticks and TBDs among high-risk groups for the first time in Greece. The study relied on a 45 closed ended question based structured questionnaire consisting of seven sections. Permanent adult residents belonging to high-risk groups from three municipalities (Veria, Naoussa, Edessa), where *Ixodes ricinus* was previously shown to predominate, were enrolled. In total 172 questionnaires were included in the study. Among high-risk group participants 23.3% reported a past tick bite. The percentage among participants reporting a past TBD diagnosis, the presence of erythema migrans and red meat allergy ranged between 1.7%-2.3%. Knowledge levels were deemed inadequate (eg. relating among others to recommended tick removal practices, tick biology, TBD knowledge and insect repellent efficacy). A past tick bite (OR: 3,825, 95% C.I: 1,798-8,139) and past information on tick bite/TBD prevention (OR: 2,288, 95% C.I: 1,163-4,498) were significant predictors of higher knowledge levels. Perception of the effectiveness of the protective practices against tick bites and their application in the field varied considerably depending on the respective practice. Age group 18-39 (OR: 2,596, 95% C.I: 0,999-6,746) and a lower perceived effectiveness of the protective practices (OR: 2,731, 95% C.I: 1,422-5,243) were significant predictors of a lower adherence to the recommended protective practices. Future health education/promotion campaigns targeting the public and high-risk groups should focus on improving knowledge levels and perceived effectiveness of recommended preventive practices by considering both potential barriers and cues to action.

PP 095

Prevalence and predictors of tick-borne pathogens in deer communities

H. Sprong[1], *M. Holding*[1]
[1]RIVM, Z&O, Bilthoven, Netherlands

Background: It is expected that varying vertebrate communities affect 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: Blood samples from ~2,400 deer from across six species were collected from across the United Kingdom. These are being analysed and typed by 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 mitochondri*, *Neoehrlichia mikurensis*, *Rickettsia* spp., *Rickettsiella* spp., *Spiroplasma ixodetis*, *Trypanosoma* spp., and Uukuniemi-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. mitochondri, 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.

PP 096

Wild carnivores are potential reservoir hosts for *Anaplasma phagocytophilum* strains pathogenic for humans

S. K. Bednarek[1], *A. S. Kruppenbacher*[1], *F. D. von Loewenich*[1]
[1]Institute of Virology, University of Mainz, Mainz, Germany

Anaplasma phagocytophilum is a Gram-negative bacterium that is transmitted by ticks of the *Ixodes ricinus* complex. It is the causative agent of granulocytic anaplasmosis primarily in humans, dogs, horses, cats, sheep, cattle and goats. Transovarial transmission of *A. phagocytophilum* is not effective in *Ixodes* spp. Therefore, the pathogen is dependent on reservoir hosts to maintain its life cycle. In Europe, the wildlife reservoir for human infection is still a matter of debate. As homologous strains occur in humans, hedgehogs and wild boar, these animals have been implicated in the transmission cycle of isolates pathogenic for humans. However, wild carnivores have not been extensively studied in this respect.

Therefore, *A. phagocytophilum* strains from 13 raccoons, 2 wolves, 1 jackal and 1 polecat were genetically characterized using multilocus sequences typing (MLST) and *ankA* gene-based typing. Nine of them had sequence types (ST 25, ST 54) and an *ankA* allele (allele number 12) identical to strains found in humans. Thus, wild carnivores should be considered as reservoir host for human infection and further studied.

PP 097

Genetic diversity of tick-borne zoonotic pathogens in ixodid ticks collected from small ruminants in Northern Pakistan

J. Zeb[1]
[1]The University of Hong Kong, School of Public Health, Hong Kong, Hong Kong

Mapping tick distribution and pathogens in unexplored areas sheds light on their importance in zoonotic and veterinary contexts. In this study, we performed a comprehensive investigation of the genetic diversity of tick and tick-borne pathogens (TBPs) detection infesting/infecting small ruminants across northern Pakistan. We collected 1,587 ixodid ticks from 600 goats and sheep, an overall tick infestation rate of 50.2 %. Notably, gender-based infestation rates were higher in female goats and sheep compared to their male counterparts. Age-wise analysis showed that the tick infestation rate was higher in older animals. This study identified 11 ixodid tick species within three genera: *Hyalomma*, *Haemaphysalis*, and *Rhipicephalus*, which were taxonomically classified using 16S rRNA and cytochrome oxidase I (cox1) molecular markers. Sequence analysis indicated that reported ticks are similar to ixodid species found across various Asian and African countries. Tick-borne pathogens were detected by amplifying 16S rRNA and citrate synthase *(gltA*) for bacterial pathogens and 18S rRNA for apicomplexan parasites. The present study reported a diverse array of TBPs in ticks from the study area, with *Rickettsia massiliae* (24.5 %) and *Theileria ovis* (16.4 %) as the most prevalent bacterial and apicomplexan pathogens. Phylogenetically, detected TBPs shared evolutionary relatedness with identical TBPs from old and new world countries. These findings highlight the presence of zoonotic TBPs in ixodid ticks from Pakistan. In addition, it also provides a foundation for future epidemiological research on ticks and TBPs, emphasizing their relevance in both zoonotic and veterinary contexts.

Keywords: Ticks, Tick-borne pathogens, Small ruminants, Khyber Pakhtunkhwa, Gilgit Baltistan, Northern Pakistan

PP 098

Comparative biochemical characterisation and inhibitory profiling of cattle tick, human, bovine and mosquito Flavin Adenine Dinucleotide sub-domains

N. Cowley[1], *R. Finn*[2], *O. Sparagano*[3], *A. Pérez de León*[4], *M. Zhang*[1]
[1]Northumbria University, Applied Sciences, Newcastle Upon Tyne, United Kingdom
[2]St George’s University International School of Medicine, Biochemistry, Newcastle Upon Tyne, United Kingdom
[3]City University of Hong Kong, Infectious Disease & Public Health, Kowloon, Hong Kong
[4]San Joaquin Valley Agricultural Research Center, Parlier, CA, United States

Southern Cattle Tick, *Rhipicephalus microplus (Rm)*, is a significant ectoparasite of tropical/sub-tropical regions, causing health detriments and severe economic losses in the cattle industry. Resistance to common acaricides via mechanisms such as increased rates of cytochrome P450 (CYP) detoxification are a growing concern. CYPs are also essential for hormone biosynthesis, requiring a single redox partner cytochrome P450 oxidoreductase (POR) facilitating electron supply from nicotinamide adenine dinucleotide phosphate (NADPH). Inhibition of POR function would affect all CYP-catalysed reactions, resulting in multi-system failure. We report the expression and biochemical characterisation of flavin adenine dinucleotide (FAD) binding domains of RmPOR and domestic cow, *Bos taurus (Bt),* in terms of kinetic parameters for NADPH and inhibitory activity of adenine nucleotide analogues, to determine differences between host/parasite POR isoforms for exploitation. Malarial mosquito, *Anopheles gambiae (An)*, and *Homo sapiens (H)* were included to extend the study value. Purified FAD domains displayed characteristic flavin spectrum (absorbance peaks at ∼379 nm/ ∼454 nm). Michaelis-Menten constants and turnover, Kcat, for NADPH were calculated as 35.98, 62.57, 66.2 and 110.18 µM plus 0.1, 0.62, 0.96 and 1.71 sec-1 respectively for RmFAD, BtFAD, AnFAD and HFAD. Half-maximal Inhibitory Concentration (IC50) values for 2”-AMP/NADP+ were 3.5 mM/90.4 µM for RM FAD; 4.7 mM/119.6 µM for BtFAD; 3.58 mM/149.7 µM for AnFAD and 14.38 mM/209.5 µM for HFAD. Comparison of kinetic/inhibitory profiles across all four species identified differences between host-parasite pairings, supporting the potential of POR as a target for rational design of safer and more potent insecticides/acaricides against CYP-mediated resistance populations.

PP 099

Role of complement on host adaptation of *Borrelia garinii* in seabirds – insights into the terrestrial and marine cycle transmission

N. Arssi[1], G. Margos[2], V. Fingerle[2], S. Hepner[2], R. E. Rollins[3], I. dos Santos[4], J. Pereira[4], V. H. Paiva[4] K. McCoy[5], I. Lopes de Carvalho[6], A. C. Norte[4], P. Kraiczy[1]
[1]Goethe-University Frankfurt, Institute for Medical Microbiology and Infection Control, Frankfurt, Germany
[2]German National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany
[3]Institute of Avian Research -Vogelwarte Helgoland-, Wilhelmshaven, Germany
[4]University of Coimbra, Department of Life Sciences, Coimbra, Portugal
[5]University of Montpellier, Montpellier, France
[6]National Institute of Health Doutor Ricardo Jorge, Lisboa, Portugal

Complement is considered a crucial factor contributing to the host-pathogen association of Lyme disease borreliae. Birds are known to play an important role in the tick-host-*Borrelia* transmission cycle and for the geographic dispersion of spirochetes. Concerning the role of complement in reservoir competence of seabirds for *Borrelia*, only fragmentary information is available. To get insight into this, we conducted serum bactericidal assays and immunofluorescence microscopy to detect deposition of activated C3 on bacteria. Serum samples from different seabird species collected at two distinct regions in Portugal including gull chicks (*Ichthyaetus audouinii* and *Larus michahellis*) and shearwater chicks (*Calonectris borealis*) as well as sera from blackbirds (*Turdus merula*) were assayed. In addition, Japanese quail (*Coturnix japonica*), and healthy blood donors were included as well. For comparative analyses, we selected at least three seabird-associated *B. garinii* isolates cultured from *Ixodes uriae* from Norway and three control strains including a *B. garinii* tick isolate from France, and two *B. afzelii* and *B. bavariensis* human isolates.

All *B. garinii* isolates analysed exhibited pronounced resistance to sera of all seabird species while *B. afzelii* and *B. bavariensis* were efficiently killed. Assaying quail serum, *B. afzelii* cells did not survive after incubation while *B. garinii* and *B. bavariensis* did not show any signs of cell destruction and immobility. As expected, activated C3 was not detected on the surface of *B. garinii* or *B. bavariensis* isolates after incubation with quail serum. In contrast, cells of *B. afzelii* were fully covered with complement components. These differences in serum resistance pattern may be attributed to the ability of *Borrelia* cells to overcome complement attack by binding serum components from these bird species. Our findings suggest that complement contributes in the avian-spirochete-tick infection cycle and in *Borrelia*-host adaptation.

PP 100

Are rodents key hosts for *Ixodes ricinus* larvae? Insights from blood meal analysis in German ticks

A. Springer[1]*, O. Bozbey*[1]*, J. Probst*[1]*, M. Komorek*[2]*, O. Kahl*[2]*, C. Strube*[1]
[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hanover, Germany
[2]tick-radar GmbH, Berlin, Germany

Ixodes ricinus is the major vector of tick-borne pathogens in large parts of Europe. Despite many field studies, the driving factors of its local abundance are not fully understood. Host availability, particularly of rodents as hosts for larvae, has been suggested to play an important role, but such data are often difficult to obtain. Thus, blood meal analysis of ticks may reveal important insights. In the frame of a study on tick density in northern Germany, ticks were collected monthly from April to July 2021-2023 at 43 locations in 13 areas. Mean questing *I. ricinus* nymphal density was 74.1/100 m^2 in 2021, 58.1/100 m^2 in 2022 and 69.2/100 m^2 in 2023, but varied considerably among sites. Nymphal density was significantly lower in coniferous than deciduous forests.

In addition, the percentage of ground covered by leaf litter as well as temperature- and moisture-related variables on the collection date and during previous seasons were significantly associated with nymphal density. Moreover, 1,441 ticks (1,047 nymphs and 394 adult ticks) from a subset of 25 locations in seven areas were subjected to blood meal analysis by PCR of the vertebrate 12S rDNA followed by Reverse Line Blotting. In the blood meal analysis, vertebrate DNA was detected in 36.6% (528/1,441) of the ticks examined. Of these, 22.2% (117/528) each had fed on birds and ungulates, 19.7% (104/528) on small rodents and 48.3% (255/528) on other mammals, while DNA from lizards was detected in 5.7% (30/528). DNA from from more than one vertebrate group was amplified in 15.8% (63/398) of nymphs and 17.7% (23/130) of adult ticks with a detectable blood meal. There was no significant difference in the host spectra of the developmental stages, i.e. between larvae and nymphs. These data suggest that small rodents play a less important role as hosts of *I. ricinus* larvae, and thus in modulating tick abundance, than previously assumed.

PP 101

New insights on *Babesia divergens* and *Ixodes ricinus* interaction: piroplasm expression of apical complex proteins during salivary gland invasion and development

H. Kpadonou[1], *S. Rakotobe*[1], *L. Simo*[1], *V. Risco-Castillo*[1]
[1]ANSES - INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, France

The apical complex is a conserved cytoskeletal structure that organizes the secretory and invasion machinery of all apicomplexan parasites. It plays a pivotal role during host cell adhesion and invasion, both in intermediate and definitive hosts. Aconoidasida species, such as *Plasmodium* and *Babesia*, share the ability to invade arthropod vector cells, where they develop and multiply. Then, they are released into the vertebrate host during the blood meal. Key factors in the salivary gland invasion process include the secretion of tubulin proteins, the major structural proteins of the apical complex. While these proteins seem to promote efficient mosquito infection by *Plasmodium*, very little is known about this mechanism in *Babesia* sp. during tick vector invasion. In order to unveil the mechanisms driving salivary gland invasion and sporozoite development, we investigated the role of α-tubulin in *Babesia divergens* development in *Ixodes ricinus* salivary glands. We established an artificial infection model by capillary feeding to infect *I. ricinus* females. Then, we recovered salivary glands from infected larvae after moulting, and we set up an immunolabeling protocol to localise *Babesia* b-tubulin expression using a rabbit polyclonal antibody. Additionally, we utilized the *Ixodes ricinus* IRE19 cell line to determine the ability of *B. divergens* merozoites to infect them, and to observe early parasite development. The results presented in the poster will shed light on the mechanisms underlying α-tubulin expression, apical complex secretion, and salivary gland invasion.

PP 102

In silico structural biology to understand tick-microbe interactions: the case study of *Midichloria mitochondrii*

G. Bellinzona[1], M. Castelli[1], S. Melis[1], L. Mottarlini[1], J. Perner[2], A. M. J. Bonvin[3], D. Sassera[1,4]
[1]University of Pavia, Pavia, Italy
[2]Biology Centre CAS, Ceske Budejovice, Czech Republic
[3]Utrecht University, Utrecht, Netherlands
[4]Policlinico San Matteo, Pavia, Italy

Recent advances in the field of in silico structural biology have opened the possibility to perform high-quality protein structures prediction with relative ease. The main algorithm responsible for this improvement, Alphafold, can be used to determine interactions between two monomers, through the extension AlphaFold Multimer. This can in turn be customized to high-throughput screening of protein-protein interactions (PPis). Such approaches could allow to explore the molecular mechanisms of interactions between ticks, their hosts and their microorganisms.

Midichloria mitochondrii is an intracellular bacterium able to colonize the mitochondria of the cells of its host, *Ixodes ricinus*. Recent research suggested a role of the bacterium in modulating the mitochondrial network in tick cells, with undisclosed effects on organelle function and host physiology. Since the molecular bases of this interaction remain elusive, we decided to use it as a benchmark to screen all theoretical protein-protein interactions (PPIs) between a microbe and a tick. We developed a pipeline to mimic a pulldown assay and used it to screen each *Midichloria* secreted protein against the tick mitochondrial proteome, testing over 110k potential PPIs. We refined our dataset based on quality scores, retaining 416 high quality PPIs, which lay the basis for the construction of the interactome. Interestingly, a *Midichloria* protein was discovered to interact with a master regulator of the host mitochondrial network and two tick proteases were found interacting with multiple proteins from the symbiont, suggesting additional interplays with the tick mechanisms for defense and protein turnover.

Broadly, our work seeks to establish a novel workflow for the study of interspecies interactions, which could include other systems, such as those involving complex interplay between pathogens, ticks and their vertebrate hosts.

PP 103

Borrelia lusitaniae and *Borrelia burgdorferi* sensu stricto are preferentially maintained by sand lizards (*Lacerta agilis*) and their immature *Ixodes ricinus* ticks, in western Poland

J. Michalik[1]*, M. Wieczorek*[2]*, R. Grochowalska*[3]*, B. Najbar*[3]*, B. Sikora*[1]
[1]Adam Mickiewicz University, Animal Morphology, Faculty of Biology, Poznań, Poland
[2]Kepler's Science Center – Nature Centre in Zielona Góra, Zielona Góra, Poland
[3]University of Zielona Góra, Institute of Biological Sciences, Zielona Góra, Poland

This study was conducted to evaluate associations of sand lizards (*Lacerta agilis*) and their larval and nymphal *Ixodes ricinus* ticks with spirochetes of the *Borrelia burgdorferi* sensu lato group. Lizards were caught by hand from April to September 2017, in three different study sites in western Poland: (i) a small village located within the boundaries of the city of Zielona Góra (ii) a gravel pit area in Żary, and (iii) a post-gravel mining area with a lot of sunlight in Nowa Sól. Animals were characterized by sex and age and examined for ticks. A total of 167 tick-infested lizards were captured, from which 1,129 feeding *I. ricinus* ticks (837 larvae and 292 nymphs) were removed. On average, one infested animal hosted 6.8 ticks. Furthermore, biopsies of the distal part of the lizard tail were taken from 131 animals. Ticks were tested individually (n=118) and in pools (n=240). The minimum infection rate (MIR) calculated for combined tick samples was 6.3% (71 out of 358), and ranged from 5.7% to 9.7% depending on the study site. Nymphs were almost five-fold more frequently infected than larvae (15.1% and 3.2%, respectively). *Borrelia lusitaniae* proved to be the most frequently identified species and accounted for 73.2% of all tick infected samples (52 out of 71), followed by *B. burgdorferi* s.s. (23%; n=17), and *B. afzelii* (2.8%; n=2). Among 27 infected larval samples, the first two species reached relatively similar infection rates (55.6% and 40.7%, respectively). Analysis of 131 biopsies of the distal part of the lizard tail, revealed that 15 (11.5%) yielded *B. burgdorferi* s.l. DNA. Among the infected animals, *B. burgdorferi* s.s. prevailed over *B. lusitaniae* (60% and 33.3%, respectively), whereas *B. afzelii* was found only in a single sample (1.6%). Our research confirms the central role of European lizards as reservoir hosts for *B. lusitaniae*. Furthermore, the comparatively high prevalence of *B. burgdorferi* s.s. found in infected ticks (23%) and lizards (60%) indicates that they may also serve as hosts for this less specialized spirochete, which in Europe is maintained both by avian and mammalian hosts. In conclusion, our findings highlight the importance of the host element in the ecology of European spirochete species belonging to the *B. burgdorferi* s.l. group.

PP 104

Agent based modeling of a vector-host-pathogen system elucidates the effects of immune selection and host specialization on antigen evolution and strain structure in *Borreliella* populations

B. Ely[1], *W. Qiu*[2]
[1]Graduate Center, City University of New York, Biology, New York, United States
[2]Hunter College, City University of New York, Biology, New York, United States

Interactions between hosts and pathogens play a key role in the evolution of antigens, with selection for the evasion of host adaptive immunity being known to drive sequence diversification. High variability in the sequence of the outer surface lipo-protein ospC of *Borreliella* species (the pathogen responsible for Lyme Disease) is observed in natural populations, with variants forming approximately 20 ospC major groups (OMG"s). Alleles within each OMG show < 1% sequence difference, while having > 15% sequence difference between groups. A lack of intermediate genotypes, as well as observed strain specific immunity in experimental immunization of mice, suggests a role for immune selection in structuring natural populations of *Borreliella*. Multiple niche polymorphism has been suggested as an alternative hypothesis, where evasion of host innate immunity drives population structure via host specialization. Here I use agent based modelling to simulate the evolution of an antigen in a vector-host-pathogen system to study the effects that selection on immune cross reactivity and host specialization have on antigen sequence evolution. Analysis of the variants within the final populations from sets of simulations show that under the condition of immune selection, final populations lack intermediate genotypes between major groups of highly similar antigen sequences. Although host specialization may play a role in the maintenance of population structure, it is not sufficient to explain ospC evolution from a set of homologous ancestral sequences to the patterns of variation observed in current natural populations. Overall, simulation results support a crucial role for immune selection in the evolution and maintenance of strain structure in *Borreliella* populations.

PP 105

Unravelling the versatility of tick antimicrobial peptides: From immunity to pathogen control and vector competence

M. Tonk-Rügen[1], *A. Cabezas-Cruz*[2]
[1]Justus Liebig University of Giessen, Institute for Insect Biotechnology, Gießen, Germany
[2]ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale Maisons-Alfort, Maisons-Alfort, France

Ticks are obligate hematophagous arthropods that serve as vectors for a wide range of pathogens, including bacteria, viruses, fungi, and protozoa. The innate immune system of ticks, particularly antimicrobial peptides (AMPs) such as defensins, plays a crucial role in modulating pathogen colonization and influencing vector competence. We have identified and characterized defensins from multiple tick species, including *Ixodes ricinus*, *Ixodes scapularis*, and *Ixodes holocyclus*, all of which exhibit broad-spectrum antimicrobial activity.

Our studies demonstrate that tick defensins possess potent antiplasmodial activity, effectively inhibiting the growth of *Plasmodium falciparum* and *Plasmodium chabaudi in vitro*, as well as in a murine malaria model. Ancestral sequence reconstruction reveals that antimicrobial activity is an ancient and conserved feature of tick defensins, suggesting evolutionary selection for broad-spectrum defense mechanisms. Furthermore, defensins from *Ixodes* species exhibit significant antibacterial and antifungal properties, particularly against Gram-positive and Gram-negative bacteria (e.g., *Staphylococcus aureus*, *Listeria grayi*, *Escherichia coli*) and phytopathogenic fungi (e.g., *Fusarium graminearum*, *Fusarium culmorum*). Notably, the γ-core motif of certain tick defensins enhances antifungal activity and abrogates mycotoxin production, emphasizing their potential applications in medical and agricultural pathogen control.

Beyond their antimicrobial functions, tick defensins are critical in shaping vector competence. The differential expression of defensins in tick tissues such as the midgut, salivary glands, and hemolymph, suggests their role in regulating pathogen transmission. Defensins in the salivary glands may influence the ability of ticks to transmit pathogens to its host, while expression in the hemolymph could contribute to systemic immune responses. These findings indicate the importance of defensins in determining the vector capacity of ticks and suggest that they are key targets for further research on vector-pathogen interactions.

Our findings highlight the importance of tick defensins in shaping vector competence and their potential application in controlling pathogens that affect human, animal, and plant pathogens. Further investigations into their interactions with pathogens and the mechanisms influencing disease transmission will provide deeper insights into their role in vector competence and the dynamics of vector-borne diseases.

PP 106

First detection of Borreliella spirochetes from the Lyme borreliosis group in hematophagous wing mites of the genus Spinturnix (Acari: Mesostigmata) parasitizing Myotis myotis and M. daubentonii bats (Vespertilionidae) in Poland

B. Sikora[1]*, B. Wodecka*[2]*, A. Szubert-Kruszyńska*[3]*, T. Postawa*[4]*, M. Dabert*[5]*, A. Taylor 5, J. Michalik* [1]
[1]Department of Animal Morphology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland
[2]Department of Genetics and Genomics, Institute of Biology, University of Szczecin, Poland
[3]Evolutionary Biology Group, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland
[4]Institute of Systematic and Evolution of Animals, Polish Academy of Sciences, Krakow, Poland
[5]Molecular Biology Techniques Laboratory, Faculty of Biology, Adam Mickiewicz University, Poland

Hematophagous mites of the genus Spinturnix are permanent ectoparasites living on bare fragments of bat's skin like wing and tail membranes, and therefore are called wing mites. They exhibit high host specificity completing their entire life cycle on one or a few closely related bat species. In this study, we tested mites for the presence of bacteria of the Borreliaceae family. Detection of spirochete DNA was carried out using two molecular markers: the flaB gene fragment and the region located between the mag and trnI genes.

Borreliella DNA was detected in 81% of 31 pools of S. myoti associated with Myotis myotis and in 76% of 17 pools of S. andegavinus collected from M. daubentonii. Sequence analysis of both markers identified five species of the genus Borreliella in individual infections of both mite species, i.e. B. carolinensis (32% of infected pools), B. spielmanii (29%), B. afzelii (18%), B. valaisiana (8%) and B. lanei (8%). Furthermore, co-infections with two species, namely B. afzelii/B.spielmanii and B. afzelii/B. americana, were detected in two pools. Apart from B. americana, five other spirochete species were detected in two bat-associated ticks of the genus Ixodes (I. ariadnae and I. vespertilionis) examined in our previous study. In conclusion, our findings provide evidence for new transmission cycles of Lyme borreliosis spirochetes involving mites of the genus Spinturnix and their cave-dwelling Myotis. In our opinion these mites parasitizing permanently bats may act as enzootic vectors for Borreliella spirochetes.

PP 107

Molecular survey of tick-borne pathogens in dogs and their ticks in France

A. Sibari[1], *D. Tahir*[2], *V. Geolier*[2], *C. Collignon*[1], *S. Dupuis*[1], *S. Favy*[3], *T. Blondel*[1], *A. Crippa*[1],
E. Ferquel[2], *M. Varloud*[1], *V. Choumet*[2]
[1]Ceva Santé Animale, , Libourne, France2
[2]Université Paris Cité, Institut Pasteur, Environnement et Risques Infectieux, Paris, France
[3]Mon Véto, Rouen, France

Tick-borne diseases (TBDs) pose significant threats to both human and animal health worldwide. In France, the incidence of tick-borne illnesses in humans and dogs is growing, thus requiring a comprehension of the diversity of tick-borne pathogens circulating in both canine hosts and tick vectors. In this study, we aimed to characterize the molecular diversity of tick-borne pathogens infecting dogs and their ticks and investigate the current tick infestations in dogs in France.

Overall, 40 veterinary practices were involved in blood and tick collections from dogs from different geographical locations in France between October 2022 and December 2023. Dogs infested with one or more ticks were recruited. A consent form was filled by their owner. Each dog was screened using a rapid test (SNAP 4Dx Plus® IDEXX) targeting 4 pathogens: Ehrlichia spp., Borrelia burgdorferi, Anaplasma spp., Dirofilaria immitis. Clinical case forms were available to fill by the practitioners for all dogs. Ticks were morphologically identified and categorized according to their stage, sex, and engorgement status. DNA extracted from canine blood samples and tick specimens was subjected to PCR amplification targeting pathogens (Babesia spp ., Borrelia s burgdorferi sensu lato., Anaplasma platys., Anaplasmataceae, Ehrlichia spp) followed by sequencing and phylogenetic analysis.

A total of 83 dogs and 165 ticks were sampled. The most common tick species collected from dogs were Dermacentor reticulatus (32.7%), I. ricinus (20%), Rhipicephalus sanguineus 18%), I. hexagonus (7.3%). The infestation intensity varied between 1 to 15 ticks/dog. Blood engorgement of D. reticulatus, I. ricinus, Rh. sanguineus and I. hexagonus was observed in 44.8%, 73.3%, 48.1% and 100% of specimens, respectively. Although the tick infestation cannot be directly linked to the concomitant TBD states of the dog, it is considered as a risk factor. Four dogs were positive on the rapid test (1 Ehrlichia, 3 Anaplasma spp.). Five dogs showed positive blood smears for Babesia. More dogs were positive when their sera were analysed by PCR for at least one of the pathogen tested. Eight dogs exhibited at least one clinical sign (e.g., hyperthermia, anorexia, haematuria) of infection potentially linked to TBDs.

This study provided valuable insights into the epidemiology of TBDs in France and emphasizes the importance of continued surveillance of these pathogens in dogs for prevention and early treatment. Our data support the role of dogs as sentinels to assess the risk of human TBD infection in a given area and demonstrate the importance of veterinarians in this One health approach.

OP 058

Overwintering, trans-stadial transmission and long persistence of the European and Siberian tick-borne encephalitis virus strains in *Ixodes persulcatus*, *Ixodes ricinus* ticks and their hybrids

O. Belova[1], *A. Polienko*[1], *G. Karganova*[1]
[1]Chumakov FSC R&D IBP RAS (Institute of Poliomyelitis), Laboratory of Biology of arboviruses, Moscow, Russian Federation

Ixodes ricinus and *I. persulcatus* ticks are the main vectors and reservoirs for tick-borne encephalitis virus (TBEV), which has three main subtypes connected with certain tick species: European (Eu) with *I. ricinus*, Siberian (Sib) and Far-Eastern - with *I. persulcatus*. In the East European Plain and Baltic countries range of these tick species overlap and form large sympatric areas, where appearance of sterile hybrids is possible. It remains unknown how overwintering of infected ticks affects further transmission of the virus. In this work, we infected larvae of *I. persulcatus*, *I. ricinus* ticks and their hybrids (from both reciprocal crossings) with Eu and Sib TBEV strains by feeding on infected mice. Engorged larvae were placed in nylon bags and were put for wintering in a foam shelter with a 30 cm leaf litter. The efficiency of TBEV acquisition before and after wintering, as well as trans-stadial transmission were analysed by RT-PCR.

The acquisition effectiveness of both TBEV strains in all experimental tick groups did not differ and exceeded 80%; the number of RNA copies of the strains was higher in a specific tick species. After wintering, in *I. ricinus* and hybrid engorged larvae, the detection of the Sib strain was significantly lower than that of Eu. Molting was observed only in *I. persulcatus* and hybrids (*I. persulcatus*♀ × *I. ricinus*♂), and the success of molting was significantly higher in infected ticks. The infection rate of these nymphs with the Sib strain was significantly higher than that of Eu. Next, *I. persulcatus* nymphs were fed on uninfected mice. Prevalence of the Sib and Eu strains in adult ticks 424 days after the second molt was similar (87.5 and 79.2%, respectively); the number of RNA copies of both strains did not differ.

For the first time, data on wintering of infected and uninfected main vectors and their hybrids, and the survival of Sib and Eu TBEV strains throughout almost the entire life cycle of these ticks was obtained.

OP 059

The role of ungulates in tick-borne pathogen epidemiology – bloodmeal duration affects *Borrelia* spp. and *Anaplasma phagocytophilum* infection status and pathogen load in *Ixodes ricinus*

A. Springer[1], *G. Özbakış-Beceriklisoy*[2], *A. K. Topp*[1], *V. Fingerle*[3], *C. Strube*[1]
[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hanover, Germany
[2]Ankara University, Faculty of Veterinary Medicine, Department of Parasitology, Anakara, Turkey
[3]National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany

The circulation of tick-borne pathogens involves vector ticks, in Europe mainly *Ixodes ricinus*, and vertebrate hosts. Wild and domestic ungulates are reservoir-incompetent for *Borrelia burgdorferi* sensu lato (s.l.), possibly even clearing the infection from ticks, while acting as reservoirs for *Anaplasma phagocytophilum*. To further elucidate the effect of an ungulate bloodmeal on *I. ricinus*-borne pathogens, *Borrelia* spp. and *A. phagocytophilum* infections in ticks from deer and cattle were investigated in relation to engorgement duration, inferred from tick dimensions. In total, 1,874 ticks were collected from wildlife. Of these, 1,535 *Ixodes* spp. from deer were tested by qPCR, with an overall prevalence of 26.3% for *Borrelia* spp. and 87.4% for *A. phagocytophilum*. Female *Ixodes* spp., but not nymphs, showed a significant decrease of *Borrelia* spp. prevalence with increasing engorgement duration. *Borrelia* spp. differentiation by Reverse Line Blot was successful for 20 samples, with *B. burgdorferi* s.l. detected in nine and *B. miyamotoi* in one tick having engorged for $<$ 48 hours, but only four *B. burgdorferi* s.l.- vs. six *B. miyamotoi*-positive ticks with a longer engorgement duration. For *Borrelia* copy numbers, a U-shaped relationship with engorgement duration was observed. A significant increase of *A. phagocytophilum* prevalence in nymphs, and of copy numbers in female ticks from deer confirmed their reservoir function. Among 101 *I. ricinus* from cattle, *Borrelia* prevalence amounted to 4.0%, whereby the *Borrelia* species was not determined. The *A. phagocytophilum* prevalence was 42.6%, without a significant effect of bloodmeal duration. In comparison, 13.8% and 11.9% of 428 questing ticks collected on the cattle pastures were *Borrelia* spp.- and *A. phagocytophilum*-positive, respectively. The fact that only partial support was found for the hypothesis of *Borrelia* elimination from ticks during a cervid bloodmeal could be driven by acquisition or replication of *B. miyamotoi*.

OP 060

Immune response to alpha-Gal: Disease biomarkers and control of tick infestations and infectious diseases

J. de la Fuente[1,2]
[1]SaBio. Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM), Ciudad Real, Spain
[2]Oklahoma State University, Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Stillwater, OK, United States

Background. Host-vector-pathogen interactions evolved as conflict and cooperation as illustrated by the α-Gal Syndrome (AGS). While anti-α-Gal IgE antibodies in response to tick bites may result in some individuals in allergic reactions to tick bites, cetuximab and/or mammalian meat consumption, IgM/IgG antibodies in response to gut microbiota with this glycan are protective against multiple pathogens. The immunity to α-Gal activates protective mechanisms associated with antibody-mediated pathogen opsonization, and B-cell, macrophage, complement and innate immunity.

Methods. Identification by sera from AGS patients but not healthy individuals and functional characterization of tick proteins associated with allergy using zebrafish and humanized mouse α-Gal-negative animal models. Identification of probiotics with α-Gal content and evaluation of protective response to α-Gal against different pathogens such as *Mycobacterium* and tick infestations in animal models. Evaluation of oral formulations combining tick protective antigen Subolesin with heat-inactivated α-Gal-positive mycobacteria against tick infestations in cattle. Frankenbacteriosis inspired by Frankenstein developed as paratransgenesis of symbiotic/commensal bacteria producing antigens involved in tick-host-pathogen interactions to mimic and compete with tick-borne pathogens and containing α-Gal for protection against tick infestations and tick-borne pathogens such as *Anaplasma phagocytophilum*.

Results. Tick proteins with and without α-Gal were identified differentially recognized by sera from AGS patients but not healthy individuals. Proteins such as metalloprotease and salivary antigen p23 were associated with allergic reactions in response to mammalian meat consumption in the animal models. However, treatment with α-Gal and α-Gal-containing probiotics induced a protective immune response against tick infestations and pathogen infection. Franken*Sphingomonas* reduced tick infestations and pathogen infection.

Conclusions. The results provide candidate antigens for developing diagnostic (disease biomarkers), treatment and preventive interventions for AGS. Probiotics with α-Gal can boost immunity against ticks and pathogens. Frankenbacteriosis may be used to control tick pathogen infection and transmission.

OP 061

Transcriptomic and proteomic insights into the longitudinal dynamics of midgut physiology in *Ixodes* sp. females

P. Kopacek[1], *S. Lu*[2]
[1]Biology Centre CAS, Institute of Parasitology, Ceske Budejovice, Czech Republic
[2]National Institute of Allergy and Infectious Diseases, Tick-Pathogen Transmission Unit, Laboratory of Bacteriology, Hamilton, MT, United States

The recent longitudinal transcriptome and proteome studies in the midgut of adult *Ixodes scapularis* (Lu et al., 2023) and *Ixodes ricinus* (Kozelková et al., submitted), respectively, provide a more detailed insight into the dynamic molecular changes that occur during development - from the unfed stage, through the slow and rapid feeding periods, to the post-feeding (pre-oviposition) stage. Given the high genetic similarity between these two tick species, it is possible to identify corresponding orthologs and map their dynamic semi-quantitative expression profiles at both the mRNA and protein levels. A manual scrutiny of molecules that have been shown or hypothesized to be involved in key physiological processes, including blood meal digestion, regulation of the proteolytic system, heme/iron metabolism, vitellogenesis or midgut immunity was performed. The aim of this presentation is to highlight the usefulness of omics-based studies and their potential for identifying targets for the development of effective tick control strategies.

(*Acknowledgement: Supported by GACR 24-10659S*)

OP 062

Insight into the tick neurosecretory system

L. Simo[1], *C. Ning*[1], *L. Abiun-Denis*[1], *S. Rakotobe*[1], *M. Vancova*[2], *R. Sima*[2], *T. Bily*[2], *J. Tyc*[2]
L. Sofrankova[3]
[1]ANSES – INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Animal Health, Maisons-Alfort, France
[2]Czech Academy of Sciences, Institute of Parasitology, Ceske Budejovice, Czech Republic
[3]University of Pavol Jozef Safarik, Kosice, Physiology, Kosice, Slovakia

Neurosecretory cells (NSC) are defined as specialized neurons that produce and release chemical messengers into the bloodstream to target distant tissues. Although, it seems that tick and insect nervous systems have developed convergently, as of today the information about tick neurosecretory system along its releasing sites remains literally a "black box". In presented work we used specific antibodies against different classes of neuropeptides to reveal completely novel lateral and medial clusters of peptidergic NSC in the anterior protocerebrum of *Ixodes ricinus* synganglion. On one hand, each of these NSC, form a rich axonal network on the dorso-lateral surface of the synganglion, while on the other hand also send their axons into nearly all internal lobes of this organ. Immunogold transmission electron microscopy confirmed that surface axons of these cells terminate within the dorsal perineurium and release the neuropeptides towards an acellular neurilemma layer, that faces the circularly fluid, the haemolymph. We also found that axon terminals of both lateral and medial NSC are sensitive to acetylcholine (ACh) neurotransmitter as they express type A and B muscarinic acetylcholine receptors, respectively. Injection of 100 nL water into unfed *I. ricinus* female haemocoel intensified the fluorescent neuropeptide signal in these axon terminals, while the same volume containing 10 μM ACh had a significantly reduced effect. Here, we hypothesize that increased fluorescence is associated with accelerated neuropeptide synthesis and subsequent transport to axon terminals as a consequence of sensing the changes in the haemolymph volume. On the other hand, a decreased fluorescent signal indicates that ACh mediates release of these neuropeptides that act as hormones for the regulation of osmoregulatory or other physiological processes. Based on these results we propose a unique model of hormonal acetylcholine-triggered released of peptide hormones in ticks.

OP 063

Functional Insights into tick-microbe interactions

L. Robbertse[1], T. Hatalova[1], V. Urbanova[1], P. Kopacek[1], R. Sima[1], O. Hajdusek[1], G. Bellinzona[2]
D. Sassera[2], J. Perner[1]
[1]Biology Centre Czech Academy of Sciences, Institute of Parasitology, České Budějovice, Czech Republic
[2]University of Pavia, Pavia, Italy

Arthropods, a highly diverse and abundant group of organisms, showcase a remarkable spectrum of symbiotic mechanisms, making them a study model for biological interactions. Ticks, too, are far from being solitary; apart from horizontally acquired microbes (: pathogens), they carry endosymbiotic bacteria, which are inherited vertically and passed from one generation to the next. Unlike insects, most tick species have digestive tracts poor in bacterial occupation. Instead, most bacterial proliferation takes place in the ovaries of tick females. The functional outcomes of such associations, however, are often not clear. *Midichloria mitochondrii,* for example, is an endosymbiotic bacterium of *Ixodes* ticks and is unique in its capacity to invade the mitochondria of some *Ixodes* species. The Array tomography and 3D reconstruction determine that the symbiont specifically occupies the cristae of tick mitochondria.

Despite its prevalence, the functional implications of this symbiotic integration remain underexplored. Recent advancements, including the generation of symbiont-free *Ixodes ricinus* ticks, have enabled a deeper investigation into the physiological role of *Midichloria*. Our studies reveal that symbiont-free females produce larvae with diminished blood-feeding success, underscoring the symbiont's critical role in host fitness. Comparative analyses between symbiont-free and wild-type ticks identified molecular changes linked to intra-mitochondrial symbiosis, highlighting a symbiont-mediated immune enhancement in the ovaries of female ticks.

Ongoing RNAi screens aim to elucidate the specific immune components involved in regulating symbiont loads, offering insights into the dynamic interplay between host and symbiont. By leveraging this novel model system, we aim to uncover fundamental molecular and cellular mechanisms underpinning the unique relationship between bacteria and eukaryotic organelles.

OP 064

The tick-pathogen anti-complement evolution dictates host skin immunomodulation for multimodal microbial transmission

C. Fernandes[1,2], A. Marcinkiewicz[1,2], A. Wang[2], J. Malfetano[2], D. Palmer[2], T. Nowak[2,3], P. Kraiczy[4]
G. Andersen[5], S. O. Kolokotronis[6], Y. P. Lin[1,2]
[1]Cummings School of Veterinary Medicine, Infectious Disease and Global Health, North Grafton, MA, United States
[2]Wadsworth Center, New York State Department of Health, Albany, NY, United States
[3]SUNY Albany, Albany, Germany
[4]University Hospital of Frankfurt, Frankfurt, Germany
[5]Aarhus University, Molecular Biology and Genetics, Aarhus, Denmark
[6]SUNY Downstate Health Sciences University, Brooklyn, NY, United States

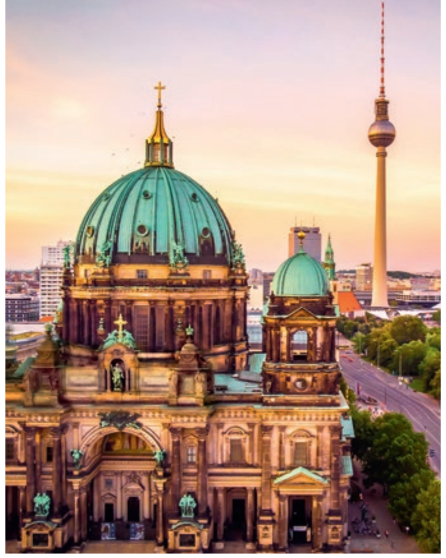
Overcoming a robust host immunity at initial infection sites is the prerequisite of pathogen transmission. For vector-borne pathogens, skin immune responses at bite sites can be suppressed by arthropod vector saliva, raising a question for the role of vector-pathogen synergy in modulating host immunity and pathogen transmission. The causative agent of Lyme disease, *Borrelia burgdorferi* (*Bb*), invades reservoir hosts through blood feeding by *Ixodes* ticks. Tick-to-host transmission of *Bb* requires naïve larvae feeding simultaneously with physically close *Bb*-infected nymphs (cofeeding-mediated transmission) or subsequently to *Bb*-persistent hosts (dissemination-mediated transmission). *Bb* produces an outer surface protein, CspZ, that binds to a complement inhibitor, factor H (FH) and FH-like protein-1 (FHL-1), evading the alternative complement pathway (AP). Similarly, ticks secrete the Salp20 protein in saliva by binding to properdin, preventing AP activation. By predicting the structure of the Salp20-properdin complex, we located the properdin-binding functions in a Salp20 loop. We then found synergy of CspZ and Salp20, conferring cofeeding- and dissemination-mediated transmission by promoting collaborative AP inactivation and *Bb* infection at bite sites. Through transcriptomic analysis, we observed the requirement of CspZ-Salp20 synergy to alleviate *Bb*-triggered inflammations at tick bite sites. We further attributed such a synergistic AP inactivation and skin immunomodulation to the Salp20 loop and the CspZ FH-binding pocket, highlighted by evolutionary analyses showing this synergy under positive Darwinian selection. This multi-disciplinary study linked CspZ-Salp20 synergy-mediated skin immunomodulation to multimodal *Bb* transmission, providing a system to elucidate the mechanisms of pathogen transmission by vector-pathogen collaborations.

OP 065

A dynamic histamine continuum shapes the tick-host interaction

L. Robbertse[1], *T. Hatalová*[1], *S. Opekar*[2], *D. Foitlová*[1], *L. Simo*[1,3], *J. Ribeiro*[4], *M. Moos*[2], *J. Perner*[1]
[1]Biological Center of the ASCR, vvi, Institute of Parasitology, Biology Centre CAS, České Budějovice, Czech Republic
[2]Biological Center of the ASCR, vvi, Institute of Entomology, Biology Centre CAS, České Budějovice, Czech Republic
[3]French National Institute for Agriculture, Food, and Environment (INRAE), Paris, France
[4]National Institute of Allergy and Infectious Diseases, Laboratory of Malaria and Vector Research, Rockville, MD, United States

Ticks are notorious vectors of various pathogens, posing growing concerns in human and veterinary medicine. Understanding the biological mechanisms that allow ticks to remain attached to their hosts for extended periods is crucial. A key immune response triggered by arthropod bites is histamine release, which induces localized inflammation, itching, and discomfort. While many biting insects escape before these effects, ticks have evolved specialized strategies to counteract histamine, allowing them to stay attached for days. Winged insects typically avoid histamine by fleeing shortly after biting, but ticks—especially slow-feeding species like *Ixodes ricinus*—remain attached to their host for days. Soft ticks, which feed quickly, utilize scavenger proteins in their saliva to bind and neutralize histamine. However, *I. ricinus* a slow-feeding tick is not endowed with such binding capacity in their saliva and, instead, exhibit a novel evolutionary strategy: histamine recycling. Our research demonstrates that this tick species is exposed to high levels of host histamine, which it ingests and transports to its salivary glands for excretion back into the host. This process reduces intra-organismal levels of host derived histamine, thereby diminishing immune-mediated impediments to tick feeding. The broader significance of this discovery lies in revealing how arthropods have evolved diverse strategies to overcome host defences. These findings have important implications for understanding tick biology, host-parasite interactions, and developing future vector control strategies.



© Alexey Fedorenko; nadiinko; RODIYAH I stock.adobe.com