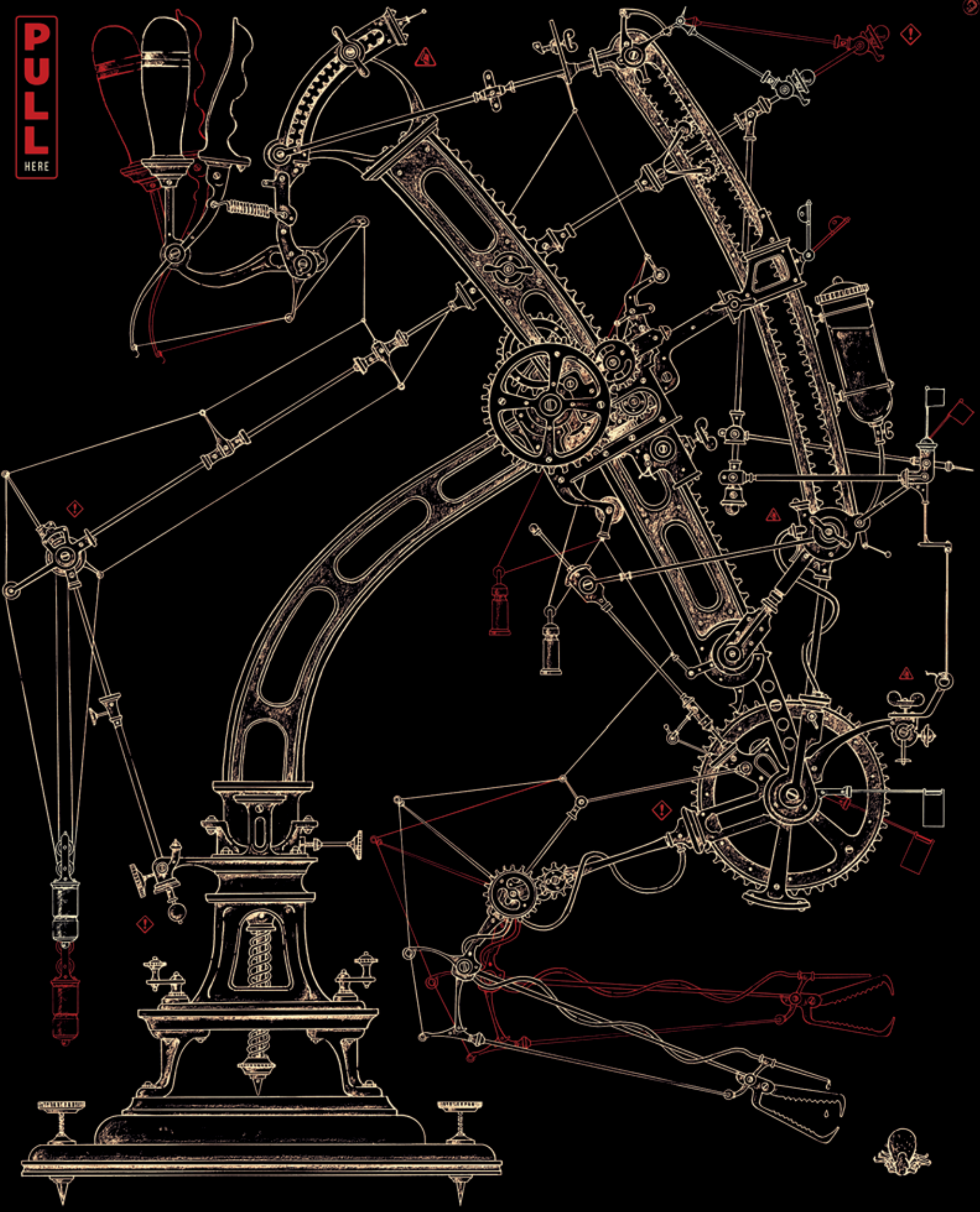


BEWARE of TICKS

REMOVE them CAREFULLY

XVth INTERNATIONAL SYMPOSIUM ON TICKS AND TICK-BORNE DISEASES

29th–31st MARCH 2023 — LEONARDO HOTEL WEIMAR

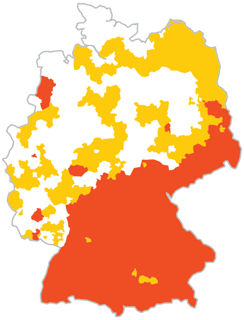


TABLE OF CONTENTS ISTTBD-XV

Scientific programme

Poster presentations

DESIGN/LAYOUT ISTTBD-XV

Layout	Conventus Congressmanagement und Marketing GmbH)
Print	printworld.com GmbH I Messering 5 I 01067 Dresden
Editorial Deadline	10 March 2023

© Layout Cover	Robert Voss

ISTTBD-XV ORGANIZATION AND IMPRINT

Date
29–31 March 2023

Hosting society
German Society for Parasitology (DGP)

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 (Berlin/DE)
Co-Editor-in-Chief of Ticks and Tick-borne Diseases

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

Prof. (a.D.) Dr. Jochen Süss
Brehm-Gedenkstätte Renthendorf
Editor-in-Chief of Ticks and Tick-borne Diseases (Renthendorf/DE)

Scientific committee
Gerhard Dobler (Bundeswehr Institute of Microbiology)
Ute Mackenstedt (Universität Hohenheim)
Jeremy Gray (University College Dublin)

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

Dear Colleagues,

It is our great pleasure to welcome you to the 15th International Symposium on Ticks and Tick-borne Diseases (ISTTBD-XV) in the beautiful town of Weimar.

After the previous symposium had to be held exclusively online in 2021, we have been really looking forward to organizing an in-person symposium again. At the same time, the benefits of online meetings are also obvious and with the extensive online exchanges of the 2021 symposium in mind, we decided to organize ISTTBD-XV as a hybrid event. We hope that the hybrid format will allow for ample personal exchange while at the same time giving participants the possibility to join and actively attend the symposium online. We would greatly appreciate hearing your feedback on this new format and hope that you will share your experiences with us, either personally during the conference, for example during the 'Talk to the organizers' session, or in the post-conference online evaluation that will be conducted by the conference project team.

We are very pleased and would like to thank you all for the record-high number of abstracts that were submitted for presentation at ISTTBD-XV. These presentations of course form the backbone of the symposium, and we regret that due to time restrictions, we could not accommodate more oral talks in the programme.

Another essential component of each symposium are the sponsors, without whom the organization would not have been possible. We sincerely thank our sponsors: Pfizer, Bavarian Nordic, Elsevier, tick-radar GmbH and Reagena for their, for their support.

We are looking forward to a very interesting scientific programme that hopefully appeals to everyone. It includes, among others, six keynote lectures, the traditional Sinnecker-Kunz-Award session for young researchers (the winner of which will receive a wonderful green glass tick created by the glass artist Wolfgang Nickel from Georgenzell, Germany) and extended poster sessions with awards for the two best posters.

The artist Robert Voss from Halle was once again responsible for the design of the symposium poster, which this year features a fantastic tick-removal machine.

We wish all ISTTBD-XV participants an exciting symposium with lots of interaction, inspiration and fun!

Yours,
Jochen Süss, Olaf Kahl, Ard Nijhof
Conference chairs, ISTTBD-XV

ISTTBD-XV PROGRAMME OVERVIEW

29.03.2023	30.03.2023			31.03.2023	
Goethesaal	Goethesaal	Forum	Belvedere	Goethesaal	Belvedere
	08:50–09:20 **Keynote 3**			**09:00–09:30** **Keynote 5**	
	09:20–10:15 **Session 3** Tick-borne Encephalitis			**09:30–10:40** **Session 5** Systematics	
	10:45–11:45 **Poster flash talks**			**11:10–11:40** **Keynote 6**	
			11:45–12:45 **Poster session 1**	**11:40–12:25** **Session 6** Tick control/ South America	**12:25–13:25** **Poster session 2**
13:00–13:20 **Opening address**				**13:25–14:30** **Session 7** Tick control	
13:20–13:50 **Keynote 1**					
13:50–15:10 **Session 1** **Eco-Epidemiology**	**13:50–14:20** **Keynote 4**			**14:30–14:50** **Awards/Closing**	
	14:20–16:00 **Session 4** Borrelia				
15:40–17:05 **Award session** Sinnecker-Kunz Award					
17:15–17:45 **Keynote 2**	**16:30–18:10** **Session A** Borrelia, Bioinformatics & Molecular diagnostics	**16:30–18:10** **Session B** Tick infection, Piroplasms, Molecular diagnostic tools, African TBPs &			
17:45–18:50 **Session 2** Tick physiology & Microbiome					
19:30–21:30 Welcome Reception/poster viewing **Belvedere**					

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

13:00–13:20	Opening address
Room	Goethesaal
12:00–13:00	Lunch snack (Belvedere)
13:20–13:50	Keynote 1
Room	Goethesaal
Chairs	Hein Sprong (Bilthoven/NL), Nathalie Boulanger (Strasbourg/FR)
13:20 O 001	Ticks and the city – the role of urbanization in the spread of tick-borne zoonoses In North America Maria Diuk-Wasser (New York, NY/US)
13:50–15:10	Session 1 – Eco-Epidemiology
Room	Goethesaal
Chairs	Hein Sprong (Bilthoven/NL), Nathalie Boulanger (Strasbourg/FR)
13:50 O 002	Host-seeking behavior and density of *Amblyomma sculptum* and *Amblyomma dubitatum* ticks in the urban area of Uberlândia, Minas Gerais, Brazil – risk assessment of human exposure Matias Pablo Juan Szabó (Uberlândia/BR)
14:00 O 003	Decline of tick abundance in the city of Hannover, Germany, over a six-year period (2017–2022) Orhan Bozbey (Hannover/DE)
14:10 O 004	High-resolution modelling of the distribution of *Ixodes ricinus* in Europe using mixed models of climate and vegetation Agustín Estrada-Peña (Zaragoza/ES)
14:20	Discussion
14:30 O 005	Winter activity of ticks in Germany – results from field studies, field plots and a veterinarian submission study Julia Probst (Hannover/DE)
14:40 O 006	Visualizing the global distribution of ticks using Google Earth Franz Rubel (Vienna/AT)
14:50 O 007	German *Ixodes inopinatus* samples may not actually represent this tick species Robert Ethan Rollins (Wilhelmshaven/DE)
15:00	Discussion
15:00–15:40	Coffee break (Belvedere)

15:40–17:05	Award session – Sinnecker-Kunz Award
Room	Goethesaal
Chairs	Christina Strube (Hannover/DE), Agustín Estrada-Peña (Zaragoza/ES)
15:40	Introduction Sinnecker-Kunz Award
15:45 O 008	Alternatives to the use of experimental animals in studies focusing on *Theileria annulata* Khawla Elati (Berlin/DE)
15:55 O 009	Variation in *Ixodes ricinus* density and pathogen prevalence within forest stands driven by vegetation and local host abundance Mats Van Gestel (Hemiksem/BE)
16:05 O 010	Metagenomic profiling of viruses identified in tick populations collected in Laos Amal Bennouna (Paris/FR)
16:15 O 011	Molecular and MALDI-TOF MS characterisation of *Hyalomma aegyptium* ticks collected from turtles and their associated microorganisms in Algeria Hanene Benyahia (Marseille/FR)
16:25 O 012	Linking *Borrelia burgdorferi* peptidoglycan and Lyme arthritis – Correlation or causation? Mecaila McClune (Blacksburg, VA/US)
16:35 O 013	Comparison of neutralising activity of vaccine-induced TBEV antibodies against 10 genetically different TBEV strains of the European subtypes Franziska Greiner (Stuttgart/DE)
16:45	Discussion

17:15–17:45 Room Chairs	Keynote 2 – Pfizer Keynote Goethesaal Lesley Bell-Sakyi (Liverpool/GB), Alejandro Cabezas-Cruz
17:15 O 014	Ticks — world‘s most dangerous vectors. How disguise and glutinous feeding facilitates immense reproductive success and tick-borne disease transmission. Daniel Sonenshine (Rockville, MD/US)
17:45	Discussion
17:50–18:55 Room Chairs	Session 2 – Tick physiology & Microbiome Goethesaal Lesley Bell-Sakyi (Liverpool/GB), Alejandro Cabezas-Cruz
17:50 O 015	Dual SIFamide neuropeptide receptors in control of *Ixodes salivary* glands Ladislav Simo (Maisons-Alfort/FR)
18:00 O 016	Molecular outsourcing of haem as a parasitic strategy of ticks Jan Perner (České Budějovice/CZ)
18:10 O 017	The Toll pathway regulates tick immune response Ondrej Hajdusek (České Budějovice/CZ)
18:20	Discussion
18:25 O 018	Functional analysis of folate biosynthesis genes of *Rickettsia monacensis* str. Humboldt via complementation assay Jianmin Zhong (Arcata, CA/US)
18:35 O 019	What is the origin of the “core” microbiome of ticks? Natalia Fernández Ruiz (Zaragoza/ES)
18:45	Discussion
19:30–21:30 Room	Welcome Reception with poster viewing Belvedere

08:50–09:20 Room Chairs	Keynote 3 Goethesaal Gerhard Dobler (Munich/DE), Martin Palus (Ceske Budejovice/CZ)
08:50 O 020	TBEV at the blood brain barrier and behind Martin Palus (České Budějovice/CZ)
09:20–10:15 Room Chairs	Session 3 – Tick-borne Encephalitis Goethesaal Gerhard Dobler (Munich/DE), Martin Palus (Ceske Budejovice/CZ)
09:20 O 021	Transmission of the tick-borne encephalitis virus by hybrids of *Ixodes ricinus* and *Ixodes persulcatus* ticks Oxana Belova (Moscow/RU)
09:30 O 022	Complete genome sequencing of tick-borne encephalitis virus reveals potential mutations in viral genome associated with disease course Samo Zakotnik (Ljubljana/SI)
09:40 O 023	TBE virus sero-prevalence in TBE highly endemic areas Gerhard Dobler (Munich/DE)
09:50 O 024	Ecology of Powassan virus transmission in North America. Saravanan Thangamani (Syracuse, NY/US)
10:00	Discussion
10:15–10:45	Coffee break (Belvedere)
10:45–11:45 Room Chairs	Poster flash talks Goethesaal Brian Stevenson (Lexington, KY/US), Maria Diuk-Wasser (New York, NY/US)
10:45 P 001	Spotted fever group rickettsiae in ticks and small mammals in grassland and forest habitats in Central Germany Charlotte Arz (Leipzig/DE)
10:50 P 002	Tick paralysis due to *Ixodes gibbosus*? – Enigmatic cases in domestic mammals from Cyprus Anastasia Diakou (Thessaloniki/GR)

10:55 P 003	Using multiple ecological niche modelling approaches to determine the geographical distribution of *Ixodes ricinus* and *Dermacentor reticulatus* in Europe Madeleine Noll (Neston/GB)
11:00 P 004	Comparison of erythema migrans in patients with or without neurologic involvement Katarina Ogrinc (Ljubljana/SI)
11:05 P 005	Prevalence of *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and Babesia spp. in questing *Ixodes ricinus* across recreational areas in England and Wales Sara Gandy (Salisbury/GB)
11:10 P 006	Tick-borne pathogens in European bison (*Bison bonasus*) from Lithuania and Poland Algimantas Paulauskas (Kaunas/LT)
11:15 P 007	Tick-borne microorganisms detected in small mammals collected in the surroundings of two villages in Styria, Austria Michiel Wijnveld (Vienna/AT)
11:20 P 008	Characterization of the primitive immune system of *Borrelia afzelii and Borrelia garinii* Margarida Ruivo (Vienna/AT)
11:25 P 009	Infestations of *Rhipicephalus* ticks – evaluations of (unique) case reports covering the last 4 years Katrin Fachet (Stuttgart/DE)
11:30 P 010	Immune-related genes in the fat body of tick *Ixodes ricinus* Veronika Urbanová (České Budějovice/CZ)
11:35 P 011	The Tick Cell Biobank – new European tick cell lines Catherine Hartley (Liverpool/GB)
11:40 P 012	Diversity of tick species carried by migratory birds coming from Africa and detection of tick-borne *Rickettsia* spp. and *Coxiella burnetii* Elisa Mancuso (Teramo/IT)
11:45–12:45 Room	Poster session 1 Belvedere
12:45–13:45	Lunch snack (Belvedere)

13:45–13:50 Room	Poster flash talks Goethesaal
13:45 P 054	Co-infection with multiple strains of *Borrelia burgdorferi* in female mice reduces maternal antibody protection in their offspring Alexandra Foley-Eby (Saskatoon/CA)
13:50–14:20 Room Chairs	Keynote 4 Goethesaal Peter Kraiczy (Frankfurt a. M./DE), Franc Strle (Ljubljana/SI)
13:50 O 025	Cerebrospinal fluid involvement in a murine model of Lyme neuroborreliosis Catherine Brissette (Grand Forks, ND/US)
14:20–16:00 Room Chairs	Session 4 – Borrelia Goethesaal Peter Kraiczy (Frankfurt a. M./DE), Franc Strle (Ljubljana/SI)
14:20 O 026	Clinically manifested reinfections with *Borrelia burgdorferi* sensu lato: Comparison of erythema migrans during primary infection and reinfection Franc Strle (Ljubljana/SI)
14:35 O 027	Transcriptional regulation of the essential ospC gene of the Lyme disease spirochete Brian Stevenson (Lexington, KY/US)
14:50 O 028	Versatile roles of PFam54 orthologs in complement inactivation of serum resistant *Borrelia bavariensis* isolates from Japan Peter Kraiczy (Frankfurt a. M./DE)
15:05	Discussion
15:15 O 029	Host immune status and host sex influence the abundance of *Borrelia burgdorferi* in host tissues and transmission to feeding ticks Cody Koloski (Saskatoon/CA)
15:25 O 030	*Borrelia garinii* – does genome type reflect host association? Gabriele Margos (Oberschleissheim/DE)
15:40 O 031	The role of hosts in shaping highly structured *Borrelia lusitaniae* populations populations Ana Cláudia Norte (Coimbra/PT)
15:50	Discussion
16:00–16:30	Coffee break (Belvedere)

16:30–18:10	Session A – Borrelia, Bioinformatics & Molecular diagnostics
Room	Goethesaal
Chairs	Catherine Brissette (Grand Forks, ND/US), Anna Moniuszko-Malinowska (Bialystok/PL)
16:30 O 032	Development and validation of a protein array for Lyme borreliosis diagnostics Olga Stukolova (Moscow/RU)
16:40 O 033	Lyme borreliosis and health care system use in Germany – a retrospective claims data analysis in 2015–2019 Gordon Brestrich (Berlin/DE)
16:50	Discussion
16:55 O 034	*Ixodes ricinus* abundance and influencing factors in northern Germany during the years 2021–2022 Andrea Springer (Hannover/DE)
17:05 O 035	Usefulness of multiomic studies in TBE and bacterial co-infection Anna Moniuszko-Malinowska (Bialystok/PL)
17:15	Discussion
17:20 O 036	Abundance in host tissues drives lifetime transmission to ticks for 11 strains of *Borrelia burgdorferi* Christopher Zinck (Saskatoon/CA)
17:30 O 037	Host-pathogen interactions and antimicrobial defenses by innate immune receptor NLRX1 during Lyme borreliosis Juselyn D. Tupik (Blacksburg, VA/US)
17:40	Discussion
17:45 O 038	PacBio (HiFi) data need several assembler for completed Borrelia plasmid reconstruction Sabrina Hepner (Oberschleissheim/DE)
17:55 O 039	Detecting RNA viruses in tick cell cultures by SISPA and nanopore sequencing Jing Jing Khoo (Liverpool/GB)
18:05	Discussion

16:30–18:10	Session B – Tick infection, Piroplasms, Molecular diagnostic tools, African TBPs & Theileria annulata
Room	Forum
Chairs	Snorre Stuen (Stavanger/NO), Annetta Zintl (Dublin/IE)
16:30 O 040	The roles of vitellogenins in Babesia-infected *Haemaphysalis longicornis* ticks Rika Umemiya-Shirafuji (Obihiro/JP)
16:40 O 041	Rickettsial determinants for tick infection and transmission Kevin Macaluso (Mobile, AL/US)
16:50	Discussion
16:55 O 042	Avian Babesia – a description of *Babesia* sp. YLG from yellow-legged gulls and its transmission by Ornithodoros maritimus Laurence Malandrin (Nantes/FR)
17:05 O 043	A rapid and economical tool for the comprehensive identification of ticks, pathogens, and hosts, and their infection dynamics Gaurav Chauhan (Worcester, MA/US)
17:15	Discussion
17:20 O 044	Transcriptomic analysis of *Amblyomma hebraeum* ticks infected with *Ehrlichia ruminantium* David Omondi (Teltow/DE)
17:30 O 045	Tick-borne pathogens and body condition of cattle in smallholder rural livestock production systems in East and West Africa Maxime Madder (Tamarin/MU)
17:40	Discussion
17:45 O 046	Secreted *Theileria annulata* effector protein Ta9 binds and potentially activates proto-oncogenic macrophage Hck Shahin Tajeri (Berlin/DE)
17:55 O 047	Effects of feeding time on occurrence and quantity of Borrelia species and *Babesia* species in ticks detached from roe deers Peter Wilhelmsson (Linköping/SE)
18:05	Discussion
18:15–18:45	Talk to the organisers
Room	Goethesaal

09:00–09:30 Room Chairs	Keynote 5 Goethesaal Lidia Chitimia-Dobler (Munich/DE), Ben Mans (Onderstepoort, Pretoria/ZA)
09:00 O 048	Tick systematics – current trends and future directions Ben Mans (Onderstepoort, Pretoria/ZA)
09:30–10:40 Room Chairs	Session 5 – Systematics Goethesaal Lidia Chitimia-Dobler (Munich/DE), Ben Mans (Onderstepoort, Pretoria/ZA)
09:30 O 049	What have we learned from the first 630 mitochondrial genomes of ticks and other Acari? Stephen Barker (St Lucia/AU)
09:45 O 050	Seventy-eight entire mitochondrial genomes and nuclear rRNA genes provide insight into the phylogeny of the hard ticks, particularly the *Haemaphysalis* species, *Africaniella transversale* and *Robertsicus elaphensis* Samuel Kelava (St Lucia/AU)
09:55 O 051	Borrelia down under – an update on Australia"s unique *Borrelia* diversity and implications for our understanding of Borrelia evolution Alexander Gofton (Canberra/AU)
10:05	Discussion
10:15 O 052	Phylogenetic relationships of the *Amblyomma cajennense* complex (Acari: Ixodidae) at mitogenomic resolution Andrea Paola Cotes Perdomo (Bo i Telemark/NO)
10:25 O 053	*Nuttalliella* in Burmese amber – Implications for tick evolution Lidia Chitimia-Dobler (Munich/DE)
10:35	Discussion
10:40–11:10	Coffee break (Belvedere)

11:10–11:40	Keynote 6
Room	Goethesaal
Chairs	Guilherme M. Klafke (Eldorado do Sul/BR), Marcelo B. Labruna (São Paulo/BR)
11:10 O 054	Reemergence of Brazilian spotted fever – the role of human-modified landscapes Marcelo B. Labruna (São Paulo/BR)
11:40–12:25	Session 6 – Tick control I – Tick control/South America
Room	Goethesaal
Chairs	Guilherme M. Klafke (Eldorado do Sul/BR), Marcelo B. Labruna (São Paulo/BR)
11:40 O 055	Phenotypic and genotypic characterization of acaricide-resistant *Rhipicephalus microplus* field isolates from Brazil Guilherme M. Klafke (Eldorado do Sul/BR)
11:50 O 056	*Metarhizium robertsii* ROS tolerant is effective against Rhipicephalus microplus on semi-field conditions combined with an anti-tick vaccine Jéssica Fiorotti (Ribeirão Preto/BR)
12:00 O 057	Deciphering immune pathways involved in cattle tick-resistance by genomic and transcriptomic data Isabel Kinney Ferreira de Miranda Santos (Ribeirão Preto/BR)
12:10	Discussion
12:25–13:25	Poster session 2
Room	Belvedere
13:25–14:30	Session 7 – Tick control II
Room	Goethesaal
Chairs	Ute Mackenstedt (Stuttgart/DE), Hans Dautel (Berlin/DE)
13:25 O 058	Dynamics of ticks and tick-borne diseases in different ecosystems in an endemic area of France Nathalie Boulanger (Strasbourg/FR)
13:35 O 059	Vector-borne disease risk – a comprehensive analysis at the human-wildlife interface Kari Hollett (Montreal/CA)
13:45	Discussion

13:50 O 060	Potassium ion channels represent a novel target to inhibit tick blood feeding and pathogen transmission Daniel Swale (Gainesville, FL/US)
14:00 O 061	Targeting canonical metabolic pathways – a novel strategy for tick control Petr Kopáček (České Budějovice/CZ)
14:10 O 062	Proteasome inhibitors effectively combat ticks as well as transmitted pathogens Daniel Sojka (České Budějovice/CZ)
14:20	Discussion
14:30–14:50 Room	Awards and closing ceremony Goethesaal
14:50–15:30	Buffett (Belvedere)

11:45–12:45	Poster session 1
Room	Belvedere
P 013	A review of canine babesiosis with an emphasis on sub-Saharan infection Andrew Leisewitz (Auburn, AL/US)
P 014	Genetic diversity of South African cattle hard-tick species (Acari: Ixodidae) Tsireledzo Goodwill Makwarela (Florida/ZA)
P 015	Avian migration as a biological highway – determining connectivity of tick-borne pathogen communities along avian migratory routes Robert Ethan Rollins (Wilhelmshaven/DE)
P 016	High Lyme borreliosis (LB) disease burden in Germany demonstrated by the estimated incidence of symptomatic LB after adjusting for under-ascertainment by public health surveillance, 2021 Frederick Angulo (Portland, OR/US)
P 017	Genomic characterisation of four new Varanid-associated *Borrelia* spp. from Australia and Indonesia Alexander Gofton (Canberra/AU)
P 018	Molecular detection of *Anaplasma phagocytophilum* in dogs and horses from Germany (2008–2021) Ingo Schäfer (Bad Kissingen/DE)
P 019	Detection of tick-borne diseases in horses from Lithuania by serological tests and blood smear microscopy Donata Mikalauskienė (Kaunas/LT)
P 020	Lyme disease seroprevalence and larvae infestation with *B. burgdorferi* in Bulgarian songbirds Iva Trifonova (Sofia/BG)
P 021	Evaluation of two commercial ELISAs for the detection of antibodies against the tick-borne pathogen *Borrelia miyamotoi* Olga Stukolova (Moscow/RU)
P 022	*Borrelia burgdorferi sensu lato* and other tick-borne pathogens in Ixodes ricinus ticks in urban green area in Prague Eva Richtrová (Prague/CZ)

P 023	Evaluation of undertesting and underdiagnosis of tick-borne encephalitis in Germany Claudius Malerczyk (Berlin/DE)
P 024	Iflaviruses in *Ixodes* spp. cell lines – a cautionary tale Lesley Bell-Sakyi (Liverpool/GB)
P 025	*Borrelia* spp. and *Anaplasma phagocytophilum* infections in ticks from urban parks of Lithuania Justina Snegiriovaitė (Kaunas/LT)
P 026	*Babesia canis* strains circulating in dogs in Lithuania Jana Radzijevskaja (Kaunas/LT)
P 027	First results of a biological control measure of a TBEV focus in Germany Alexander Lindau (Stuttgart/DE)
P 028	Hybrid capture-based next generation sequencing of *Borrelia* in the United Kingdom Daniel Carter (Porton Down/GB)
P 029	Erythema migrans in splenectomised patients Katarina Ogrinc (Ljubljana/SI)
P 030	Influence of trails on vertebrate and tick communities in a preserved area of Atlantic Forest in southern Brazil Matias Pablo Juan Szabó (Uberlândia/BR)
P 031	Automating of an ex vivo feeding system for tick research David Hartmann (České Budějovice/CZ)
P 032	Seroprevalence of tick-borne encephalitis (TBE) virus antibodies in wild rodents from two natural TBE foci in Bavaria, Germany Philipp Johannes Brandenburg (Leipzig/DE)
P 033	Serological survey on canine and equine anaplasmosis and borreliosis in animals in contact with vulnerable people in Italy Donato Traversa (Teramo/IT)
P 034	Assessing temporal changes in microbial communities in *Hyalomma dromedarii* collected from camels in the UAE using high-throughput sequencing Nighat Perveen (Al Ain/AE)

P 035	Comparison of bacterial community between bisexual and parthenogenetic the Asian longhorn tick, *Haemaphysalis longicornis* Seoyul Hwang, Donghun Kim (Sangju si/KR)
P 036	*Neoehrlichia* in small mammals and ticks from Hainich national park Anna Obiegala (Leipzig/DE)
P 037	Molecular characterization of feline *Anaplasma phagoc*philum strains by *ankA* gene and multilocus sequence typing Friederike D. von Loewenich (Mainz/DE)
P 038	Detection of tick-borne encephalitis virus antibodies in sera of forestry workers, hunters and persons at risk in Mecklenburg-Western-Pomerania Silvius Frimmel, Tobias Schwarz (Rostock/DE), Gerhard Dobler (Munich/DE)
P 039	New tick-borne encephalitis virus isolates from North-Eastern Germany and Western Poland Nina Król (Leipzig/DE)
P 040	MLST analysis of bird-related Lyme diseases borreliae in Japan Ai Takano (Yamaguchi City/JP)
P 041	Clade C aspartyl proteases in *Babesia divergens* and their validation as novel drug targets Pavla Šnebergerová (České Budějovice/CZ)
P 042	Food-borne encephalitis and other modes of infection – a systematic review Elisa Martello (Nottingham/GB)
P 043	Preliminary results from the burden of Lyme disease (BOLD) study – a prospective active surveillance study at primary care practices in endemic regions of six European countries Alexandra Loew-Baselli (Vienna/DE)
P 044	Clinical data on reinfections with *Borrelia burgdorferi* sensu lato Franc Strle (Ljubljana/SI)
P 045	Molecular detection of ricketsia in ticks collected from big-eared opossum (*Didelphis aurita*) in forest parks in the metropolitan region of São Paulo, Brazil Michellin Pereira de Albuquerque (São Paulo/BR)
P 046	Characterization of tick species and tick-transmitted protozoa and bacteria in wild ruminants in a French zoological park Laurence Malandrin (Nantes/FR)

P 047	A metagenomic sequencing panel using nanopore reveals circulating pathogens in UK wildlife Mia White (Salisbury/GB)
P 048	Asymptomatic carriers of equine piroplasmosis – prevalence differences between 4 French regions and low genetic diversity of *Theileria equi* and *Babesia caballi* Laurence Malandrin (Nantes/FR)
P 049	Tracking the emergence of ticks and tick-borne diseases through community-engaged tick surveillance Saravanan Thangamani (Syracuse, NY/US)
P 050	Does it work? effects of permethrin-treated uniforms on tick submissions to a military passive tick surveillance program Robyn M. Nadolny (Aberdeen Proving Ground, MD/US)
P 051	Ornithological and molecular evidence of a reproducing *Hyalomma rufipes* population under continental climate in Europe Gergő Keve (Budapest/HU)
P 052	Infection of free-living ungulates and feeding ticks from south-western Slovakia with Piroplasmida and *Anaplasma phagocytophilum* D. Selyemová (Bratislava/SK)
P 053	Fitness of mCherry reporter tick-borne encephalitis virus in tick experimental models Juraj Koči (Bratislava/SK)
P 078	Seroprevalence of *Rickettsia conorii* in dogs living in Northeastern Italy Simone Morelli (Teramo/IT)
	Study on ticks for infection with *Borrelia burgdorferi* sensu lato and the impact of two climatic factors Teodora Gladnishka (Sofia/BG)

12:25–13:25 Poster session 2
Room Belvedere

P 055 Immunofluorescence analysis reveals no increased seroprevalence of anti *Bartonella schoenbuchensis* IgG antibodies in German forest workers
Volkhard Kempf (Frankfurt a. M./DE)

P 056 Seroprevalence of vector-borne relapsing fever in Kenya – a retrospective study
Peter Kraiczy (Frankfurt a. M./DE)

P 058 High disease burden of Tick-borne Encephalitis (TBE) virus infection in Latvia despite availability of FSME-IMMUN, a highly effective TBE vaccine, 2018–2020: Need to increase TBE vaccine uptake
Frederick Angulo (Portland, OR/US)

P 059 Metatranscriptomics uncovers diverse tick-borne bacteria, protozoa, and viruses in Australian ticks
Alexander Gofton (Canberra/AU)

P 060 Variant sequence analyses of *Anaplasma phagocytophilum* for *16S* rRNA and *msp4* in positive dog and horse samples from Germany
Susanne Fischer (Greifswald – Insel Riems/DE)

P 061 Effect of forest structure and landscape parameters on *Ixodes ricinus* tick abundance and infectivity
Kateřina Kybicová (Prague/CZ)

P 062 Identification of Lyme neuroborreliosis outside of Hokkaido, Japan
Ai Takano (Yamaguchi City/JP)

P 063 Building the generic roadmap for “Vector Transmission Control”
Madeline Newman (London/UK)

P 064 Importance of individual reference values in Lyme antibody testing
Elena Volokhina (Oss/NL)

P 065 Detection of *Babesia canis* in *Dermacentor reticulatus* from Germany
Andrea Springer (Hannover/DE)

P 066 Seasonal variation of *Neoehrlichia mikurensis*, *Borrelia burgdorferi* s.l. and TBEV related to the effect of climate change in Mandal, Norway
Andrea Paola Cotes Perdomo (Bo i Telemark/NO)

P 067 Outdoor worker knowledge on ticks and Lyme disease across demographics
Erica Fellin (Montreal/CA)

P 068	Diversity of ticks associated with wild animals in Cameroon Lidia Chitimia-Dobler (Munich/DE)
P 069	CCHFV and RVFV antibodies in cattle from Mozambique – closing a knowledge gap in southern Africa Ansgar Schulz (Greifswald/DE)
P 070	Molecular detection of zoonotic bacteria in ticks parasitizing cats Miglė Razgūnaitė (Kaunas/LT)
P 071	Microbiota of the brown dog tick *Rhipicephalus sanguineus* s.l. (*Acari: Ixodidae*) from Israel Aysegul Taylan Ozkan (Ankara/TR)
P 072	Enumeration of Cytokine secreting cells for the diagnosis of Lyme disease Karin Lukas (Regensburg/DE)
P 073	The tick-host interface researched through the comparative in vivo and ex vivo feeding of *Ixodes ricinus* ticks Jan Perner (České Budějovice/CZ)
P 074	Danger comes from a tree? – Pathogens detected in *Ixodes ricinus* feeding on red squirrels (*Sciurus vulgaris*) from parks in Warsaw Dorota Dwużnik-Szarek (Warsaw/PL)
P 075	Detection of Phenuiviridae, Chuiviridae members and a novel Quaranjavirus in hard ticks from Danube Delta Amal Bennouna (Paris/FR)
P 076	Molecular detection of *Borrelia* in *Ornithodoros Cavernicolous* collected in Araguapaz-Go, Brazil Mayara Garcia Polli (Uberlândia/BR)
P 077	Global distribution and climate adaptation of Ixodes trianguliceps Franz Rubel (Vienna/AT)
P 079	Membrane feeding as a tool for production of “apo-symbiotic” Ixodes ricinus ticks Tereza Hatalová (České Budějovice/CZ)
P 080	Elusive Borrelia circulating in soft ticks infesting coastal birds in Portugal Ana Cláudia Norte (Coimbra/PT)

P 081	Microbial composition in *Hyalomma anatolicum* collected from livestock in the United Arab Emirates using next-generation sequencing Mohammad Ali Al-Deeb (Al Ain/AE)
P 082	First molecular detection of multiple vector borne diseases in pet dogs and cats of Hongkong Sabir Hussain (Kowloon/HK)
P 083	One health approach in research, diagnostic and xenodiagnostic of tick-borne diseases – holistic model from Serbia Pavle Banović (Novi Sad/RS)
P 084	Affinity-based proteomic mapping of lipid hydrolases in the midgut of hard tick *Ixodes ricinus* Tereza Kozelková (České Budějovice/CZ)
P 085	Passive surveillance on exotic tick species in Sweden – results from 2019–2022 Giulio Grandi (Uppsala/SE)
P 086	A systematic review on the prevalence of tick-borne encephalitis infection in milk and milk products in Europe Elisa Martello (Nottingham/GB)
P 087	Molecular screening of *Borrelia miyamotoi* and tick-borne Encephalitis Virus in ixodid ticks in urban green areas in Serbia Gorana Veinovic (Belgrade/RS)
P 088	Experimental infection with a novel *Babesia sp.* – Evaluation of pathogenicity in healthy and immunosuppressed goats Mehmet Can Ulucesme (Elazig/TR)
P 089	Citizen science tick monitoring program to reveal emergence of *Hyalomma* ticks and potential pathogens in Hungary Éva Szabó (Budapest/HU)
P 090	Comparative blocking efficacy of two ectoparasiticides against the acquisition of *Borrelia burgdorferi* by Ixodes ricinus and Ixodes scapularis ticks in a canine ex vivo model Djamel Tahir (Paris/FR)
P 091	Resources for ticks and tick pathogens at the eukaryotic pathogen, vector and host informatics resources (VEuPathDB.org) Sarah Kelly (London/GB)

P 092	Genetic characterization of a *Rickettsia parkeri* strain in endemic areas of the coast of São Paulo state, Brazil Michellin Pereira de Albuquerque (São Paulo/BR)
P 093	Detection of *Anaplasma* spp., *Ehrlichia* spp., and *Neoehrlichia* spp. in *Ixodes granulatus* from wild animals in Kinmen County, an offshore island of Taiwan Chia-Yi Lin (Taipei/TW)
P 094	Specificity of MurE and the role of ornithine in pathogenesis of the Lyme disease spirochete *B. burgdorferi* Brittany Hart (Blacksburg, VA/US)
P 095	Efficacy and safety of pharmacological treatments for Lyme neuroborreliosis: Update of a systematic review Rick Dersch (Freiburg/DE)
P 096	*R. sanguineus* from Hungarian dogs – identification and detection of tick-borne pathogens Sajjad Ghodrati (Brno/CZ)
P 097	High diversity of tick borne agents in ticks collected from farm animals in Vrbovce (Western Slovakia) V. Rusňáková Tarageľová (Bratislava/SK)
P 097	Antibody-mediated blunting of tick salivary enzymatic “cutlery” D. Foitlová (České Budějovice/CZ)

We thank all industrial partners for supporting ISTTBD-XV.

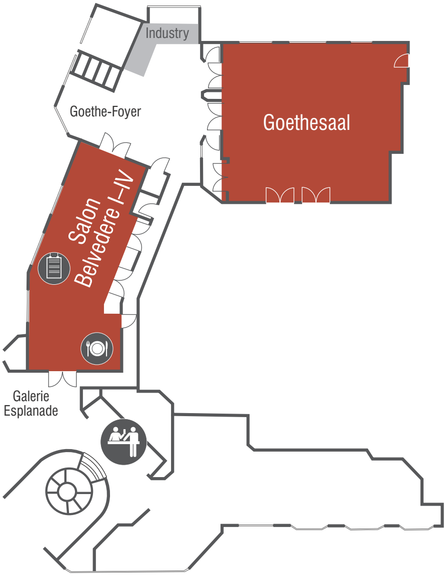
Platinum sponsor
Pfizer Deutschland GmbH

Bronz sponsor
Bavarian Nordic A/S

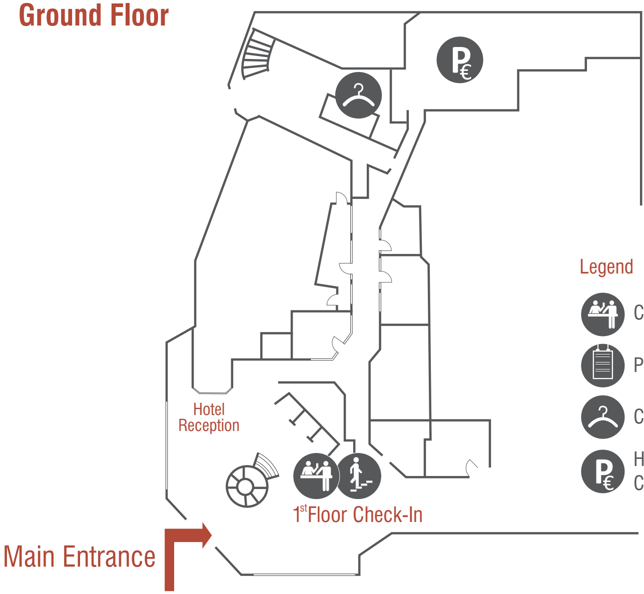
Further Sponsors
Elsevier B.V.
Tick-radar Gmbh
Reagena

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, 29 March

The organisers welcome all participants of the conference in the industrial exhibition area. Meet colleagues and other participants while enjoying snacks and beverages.

Time	19:30
Place	Belvedere & Foyer

Gala dinner I Thursday, 30 March

We invite you to join a memorable evening at the “Villa Haar” in Weimar. Celebrate the return of the 15th 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.

DJ Micky will accompany the evening and maybe even encourage you to dance…

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.

pictures: Rawpixel.com I Fotolia.com/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 takes around 14 to 20 min.

Certification

The conference/congress is accredited by the “ATF” (Akademie für tierärztliche Fortbildung der Bundestierärztekammer e. V.).

Certificate of attendance

Certificates of attendance will be send 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 also select the two best poster awards. A jury will select the best presentations given during the Sinnecker Kunz Session for early career researchers.

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 and the Forum

The media check-in for uploading your presentation is located in the back of the plenary Goethesaal and Forum when in use. (please follow the signsposting). 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 session

Two dedicated poster sessions will be held during the ISTTBD-XV – on Thursday from 11:45–12:45 and on Friday from 12:25–13:25. Presenting authors are requested to be available at their posters to present. 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 to 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.

Poster Session 1 I Thursday, 30 March I 11:45–12:45

All posters from Session 1 have to be attached by Wednesday, 29 March 13:00 and have to be removed by 16:00 on Friday, 31 March.

Poster Session 2 I Friday, 31 March I 12:25–13:25

All posters from Session 2 have to be attached by Wednesday, 29 March 13:00 and have to be removed by 16:00 on Friday, 31 March.

Sinnecker Kunz Award

The Sinnecker Kunz Award for early-career researchers (ECRs) will be presented during the 15th International Symposium on Ticks and Tick-borne Diseases (ISTTBD-XV) in 2023. The aim of the award is to acknowledge distinguished achievements by ECRs.
The award is named in honour of the late 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.

ISTTBD-XV GENERAL INFORMATION FOR ONLINE PARTICIPATION

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 anytime 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-XV.

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-viewing-browsing-and-apps.

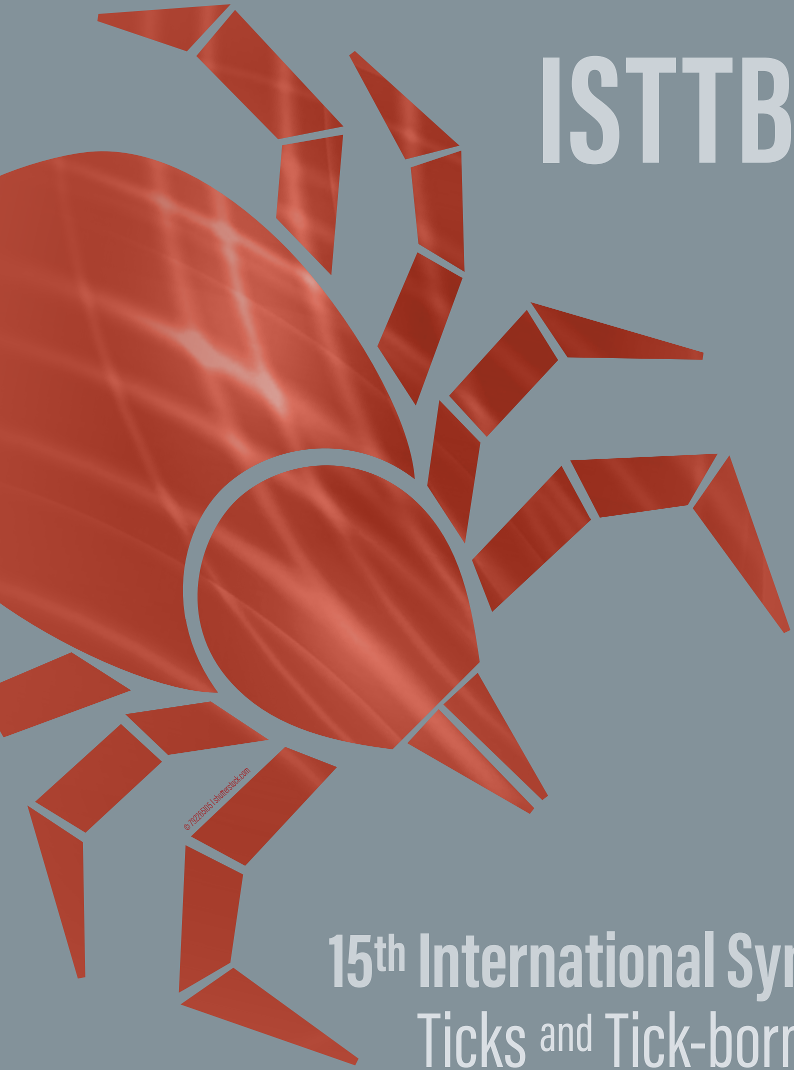
Procedure of Keynote lectures, scientific sessions and poster sessions

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.

ISTTBD-XV



© 792265105 | shutterstock.com

15th International Symposium on Ticks and Tick-borne Diseases

ABSTRACTS

Order analogous to how the abstracts appear
in the programme

O 001

Ticks and the city – the role of urbanization in the spread of tick-borne zoonoses In North America

M. Diuk-Wasser[1]
[1]Columbia University, New York, NY, United States

The emergence of tick-borne pathogens in North America is driven by the geographical expansion of human-biting ixodid tick species, in particular *Ixodes scapularis*, *Amblyomma americanum,* and *Dermacentor variabilis*, collectively serving as vectors for more than 15 human pathogens. In addition, *Haemaphysalis longicornis* was first reported in the U.S. in 2017 and has since been detected across 15 states. While important vector in its original Asian range, it not yet been implicated in transmission of native pathogens. The expansion of these tick vectors has been attributed to changes in climate, land use, and the abundance of key hosts. To examine the role of urban areas as new frontiers for tick invasion, we investigated the drivers of tick spread across urban parks and spillover into residential neighborhoods on Staten Island, a borough in New York City. Connectivity of forested land cover and the presence of white-tailed deer (reproductive host) enhanced the establishment of all tick species across Staten Island parks. The amount and configuration of canopy cover immediately surrounding residential yards strongly predicted the presence of *I. scapularis* and *A. americanum*, while there was a protective effect of fencing. The numbers of days deer were present in the yard predicted *I. scapularis* presence while those for raccoons predicted *A. americanum*. The lack of significant predictors for *H. longicornis* is consistent with its wide habitat associations and highly mobile meso- and large mammalian hosts. Our results challenge assumptions that biodiversity loss in human-modified areas increase risk for tick-borne diseases. Instead, we found many residential sites had higher species richness and higher abundance of low quality "dilution" hosts such as opossums than paired forested parks. We highlight the importance of disentangling the mechanisms mediating tick-borne disease hazard and we provides specific recommendations for reducing urban tick-borne disease risk.

O 002

Host-seeking behavior and density of *Amblyomma sculptum* and *Amblyomma dubitatum* ticks in the urban area of Uberlândia, Minas Gerais, Brazil – risk assessment of human exposure

M. P. J. Szabó[1,2], *C. Queiroz*[2], *R. Vieira*[3], *V. Rodrigues*[2], *M. M. Martins*[2], *A. Suzin*[2], *L. Rezende*[2]
A. C. Sousa[2], *V. Ramos*[2], *F. Muraro*[2], *L. Keocheguerian*[2]
[1]Universidade Federal de Uberlândia, Veterinary Pathology, Uberlândia, Brazil
[2]Universidade Federal de Uberlândia, Laboratório de Ixodologia, Uberlândia, Brazil
[3]Universidade Federal Fluminense, Niterói, Brazil

Introduction: The ticks *Amblyomma dubitatum* and *Amblyomma sculptum* in Brazil are in sympatry when kept by capybaras (*Hydrochoerus hydrochaeris*). Infestations with these ticks are high in anthropized areas and in a few areas related to the occurrence of Brazilian spotted fever (BSF). The *A. sculptum* nymph is considered the main vector of the BSF bacterium *Rickettsia rickettsii* and the proportions between the two tick species and their numbers have been shown to influence the risk of BSF.

Objectives: To assess the risk of human exposure, we herein describe the density, host-seeking behavior, and proportion among these tick species in ten capybara sites in the urban area of Uberlândia, Brazil.

Methods: Sampling units of 400 m^2 and four tick collection techniques were used at each site in three seasons.

Results: Overall, 52,953 ticks were collected, 83.4% were *A. sculptum* (1,523 adults, 10,545 nymphs and 32,104 larvae) and 16.6% *A. dubitatum* (464 adults, 2,153 nymphs and 6,164 larvae). The highest number of ticks collected was of the *A. sculptum* species, but the prevalence of the species varied among the ten study sites and according to the season of the year. The mean annual tick density was 4.4/m^2, (range 0.03-31.8 ticks/m^2). Irrespective of species, ambush host-seeking behavior predominated for larvae and hunter for nymphs and adults. A significantly higher proportion of *A. sculptum* larvae and nymphs were in ambush than *A. dubitatum*. Adults and nymphs of *A. sculptum* were significantly more attracted to humans than those of *A. dubitatum*. Conversely, larvae of *A. dubitatum* were significantly more attracted to humans.

Conclusions: Humans moving through the *A. sculptum* and *A. dubitatum* hazardous area will have almost exclusive exposure to *A. sculptum* larvae and/or nymphs. When in a stationary position, humans are more attractive to adults and especially to *A. sculptum* nymphs, but also to *A. dubitatum* larvae.

Financial support: CAPES, CNPq, FAPEMIG

O 003

Decline of tick abundance in the city of Hannover, Germany, over a six-year period (2017–2022)

O. Bozbey[1]*, A. K. Topp*[1]*, A. Glass*[1]*, K. Schütte*[1,2]*, F. Brandes*[2]*, M. Reuschel*[3]*, M. Fehl*[3]*, A. Springer*[1]
C. Strube[1]
[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hannover, Germany
[2]Wildlife Rescue and Conservation Center, Sachsenhagen, Germany
[3]University of Veterinary Medicine Hannover, Department of Small Mammal, Reptile and Avian Diseases, Hannover, Germany

Introduction: *Ixodes ricinus*, the most common tick species in Germany, serves as a vector of various pathogens. In order to adequately assess infection risk, not only studies on pathogen prevalence but also on tick abundance are important, especially in the light of changing climatic conditions.
Objectives: We aimed to investigate changes in tick abundance in the city of Hannover, Germany, over a six-year period (2017–2022).
Materials & Methods: Ten recreation sites in different habitats in the city of Hannover, the capital of the federal state Lower Saxony, were investigated from April to October of each year using the flagging method. A total area of 100 m^2 was sampled per month and site, with 50 m^2 flagged in the first and second half of each month. In addition, various climatic and environmental factors were recorded to analyse their influence on tick density. In addition, 498 hedgehogs (*Erinaceus europaeus*) from Lower Saxony were investigated for tick infestation from 2018 to 2021.
Results: In the flagging study, average tick density decreased from 25 ticks/100 m^2 in 2017 (range: 0-167) to 6 ticks/100 m^2 in 2021 (range: 0-48) and 7 ticks/100 m^2 in 2022 (range: 0-41). An autumn peak was not observed in Hannover since 2019. In addition to climatic variables at the time of sampling, cumulative precipitation during the preceding two years was identified as a significant predictor of tick density by generalized linear mixed modelling. In hedgehogs, overall prevalence of tick infestation was 86.5% (*I. hexagonus*: 66.7%, *I. ricinus:* 61.6%). Statistical modelling showed a significantly lower infestation rate with *I. ricinus* nymphs in hedgehogs in 2020 and 2021 compared to 2018 when accounting for monthly differences.
Conclusion: A decline in tick abundance was observed in the flagging as well as in the hedgehog dataset. In order to investigate whether tick populations in Hanover will increase again or continue to decrease, the flagging study will be continued in 2023.

O 004

High-resolution modelling of the distribution of *Ixodes ricinus* in Europe using mixed models of climate and vegetation

A. Estrada-Peña[1]*, N. Fernández Ruiz*[1]
[1]University of Zaragoza, Animal Health, Zaragoza, Spain

Question: Modelling approaches become popular as a possible source of information regarding hazard by tick-borne pathogens in humans. The so-called species environmental modelling uses data on the distribution of a tick to obtain an estimation of its probability of presence in an area. Other than the significant unreliabilities in published models resulting from a poor understanding of the rules governing these methods, interpolated climate (the most common source for modelling tick distribution) has a coarse resolution. The increase of resolution generates an increase of incertitude in the raw data used for modelling.

Methods: We obtained satellite imagery at 4 km resolution at 16 days intervals, gaps because snow, clouds or ice removed, to obtain the source of weather data (years 2001–2021) for mapping the weather suitability for *Ixodes ricinus* in Europe. We used a harmonic regression to obtain the explanatory variables. Using a large dataset of more than 11,000 accurately geo-referenced records of the tick, we evaluated the vegetation withwhich the tick is associated, using the pan-European CORINE dataset (year 2020), to weight the weather suitability with the vegetation. Other datasets were also evaluated aiming to an improved habitat classification over the basic CORINE.

Results: We obtained a European map at high resolution displaying the probability of presence of permanent populations of *I. ricinus* in the target territory. The agreement between the recorded and the predicted distribution of the tick is 100% for CORINE, with a resolution of about 25 ha. Other datasets of vegetation in Europe produced poorer results.

Conclusions: New approaches including vegetation, the presence of hosts and their relative importance, should be explored for generation of high resolution maps aimed to prevention evaluation of impact by ticks on humans.

O 005

Winter activity of ticks in Germany – results from field studies, field plots and a veterinarian submission study

J. Probst[1]*, A. Springer*[1]*, O. Kahl*[2]*, M. Bröker*[3]*, C. Strube*[1]
[1]Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Hannover, Germany
[2]tick-radar GmbH, Berlin, Germany
[3]Global Health Press, Marburg, Germany

In Germany, the two most important tick species are *Ixodes ricinus* and *Dermacentor reticulatus*, the latter of which has recently expanded its range across the country. While *I. ricinus*, the vector of the agents of Lyme borreliosis and granulocytic anaplasmosis, was rarely found during the colder months in the past, *D. reticulatus*, the vector of potentially fatal canine babesiosis, is known to be more cold tolerant.

We aimed to provide an update on the seasonal activity pattern and a possible winter activity of both tick species in Germany in times of climate change to assess whether current recommendations on tick control in dogs and cats might need to be adapted to changing conditions.

Three different approaches were used – Nine field collection sites were regularly sampled via the flagging method from April 2020 to April 2022, while tick activity was monitored in quasi-natural field plots three times a week. During a nationwide submission study from May 2020 to June 2021, veterinarians sent in ticks collected from cats and dogs.

In the flagging study, an average of two *I. ricinus* (range: 0-17) and 23 *D. reticulatus* (range: 0-62) were collected per 100 m^2 between December 2020 and February 2021. In the winter period 2021/22, one *I. ricinus* (range: 0-5) and three *D. reticulatus* (range: 0-5) were flagged on average. Within the tick plots, *I. ricinus* activity started in February 2021 with 4.6% (16/350) active ticks and a mean daily winter activity of 1.1% (4/350) in 2021/22. Mean daily winter activity of *D. reticulatus* amounted to 20% (20/100) in 2020/21 and 14.7% (22/150) in 2021/22. Of the 19,514 ticks received within the submission study, 211 *I. ricinus* and 324 *D. reticulatus* were collected from December 2020 to February 2021.

All three study approaches demonstrated winter activity of both tick species. To protect dogs and cats with outdoor access from ticks and tick-borne diseases, effective year-round tick control is strongly recommended.

O 006

Visualizing the global distribution of ticks using Google Earth

F. Rubel[1], *K. Brugger*[2]
[1]University of Veterinary Medicine Vienna, Vienna, Austria
[2]Austrian National Public Health Institute, Competence Center for Climate and Health, Vienna, Austria

Digital maps, particularly displayed on virtual globes such as Google Earth, will represent the most important source of geographical knowledge in the future. The use of Google Earth in teaching at schools and universities is now common practice. As the first result of a series of digital tick maps, the global distribution of *Dermacentor reticulatus* is shown. In the Google Earth application, the following maps can be selected – a historical expert map, a current expert map, a predicted habitat suitability map, a combined expert-habitat suitability map (considered to be the best representation of the current distribution of *D. reticulatus*), and a map of rasterized tick locations. It will be demonstrated how users can overlay these maps according to their own requirements or combine it with other Google Earth content such as climate maps or photos of tick locations. High-resolution city maps of Bilbao (Spain), Grenoble (France), Berlin (Germany), Wroclaw (Poland), Budapest (Hungary), Bucharest (Romania), and Tomsk (Russia) demonstrate the urban distribution of *D. reticulatus* in public parks, fallow land, and recreational areas. New tick maps that are currently being developed will also be presented. This includes maps of the global distribution of *Ixodes ricinus*, *Ixodes persulcatus*, *Ixodes trianguliceps* and *Hyalomma aegyptium*. All maps and associated content have been integrated into Keyhole Markup Language (KML) files and can be used with the free Google Earth application by scientists from various disciplines, students, and lay people interested in the geographical distribution of ticks.

O 007

German *Ixodes inopinatus* samples may not actually represent this tick species

R. E. Rollins[1], *A. Brachmann*[2], *S. Krebs*[3], *G. Margos*[4], *N. J. Dingemanse*[5], *A. Laatamna*[6]
N. Reghaissia[7], *D. Metzler*[8], *V. Fingerle*[4], *N. S. Becker*[8], *L. Chitimia-Dobler*[9]
[1]Institute of Avian Research, Wilhelmshaven, Germany
[2]LMU Munich, Genetics, Munich, Germany
[3]LMU Munich, Gene Center, Munich, Germany
[4]Bavarian Food and Health Safety Authority, National Reference Center for Borrelia, Oberschleißheim, Germany
[5]LMU Munich, Behavioral Ecology Group, Munich, Germany
[6]University of Djelfa, Faculty of Nature and Life Sciences, Djelfa, Algeria
[7]University of Souk Ahras, Laboratory of Sciences and Living Techniques, Souk Ahras, Algeria
[8]LMU Munich, Division of Evolutionary Biology, Munich, Germany
[9]Bundeswehr Institute of Microbiology, Munich, Germany

Ticks rival mosquitoes as one of the most important vectors of human and animal pathogens worldwide. Even so, many questions remain unanswered regarding the taxonomy and biology of these important arthropods. With the advent of sequencing methodologies, research has been able to start understanding the evolutionary history of even closely related tick species. In Europe, *I. ricinus* is the most important vector of many tick-borne pathogens. *Ixodes inopinatus* is described as a sister species and highly similar to *I. ricinus*, but many aspects of this tick's biology remain unknown especially outside the Mediterranean region where it was originally described. Between 2018–2019, 37 larvae and nymphs were collected from great tits (*Parus major*) in southern Germany and later morphologically identified as *I. inopinatus*. As this was potentially a novel host for *I. inopinatus*, this project aimed to molecularly confirm if these samples truly represented *I. inopinatus*. Utilizing both molecular markers and whole genomes sequencing we were able to determine that German samples identified as *I. inopinatus* instead represent *I. ricinus* and most likely are not *I. inopinatus* or *I. ricinus*/*I. inopinatus* hybrids. Whole genome sequencing of assured *I. ricinus* and *I. inopinatus* samples further shows that the entire mitochondrial genome is not sufficient in delineating *I. ricinus* and *I. inopinatus* showing that only through using nuclear markers can these species be reliably separated. The results presented here bring into question whether *I. inopinatus* exists in Germany and opens the question regarding the geographic extent of this species. Furthermore, the presented study shows the power of genetic based studies in answering open questions regarding tick taxonomy and population dynamics even when closely related species are studied.

O 008

Alternatives to the use of experimental animals in studies focusing on *Theileria annulata*

K. Elati[1,2,3]*, E. Zweygarth*[1]*, M. Moez*[2]*, M. A. Darghouth*[2]*, P. Hoffmann-Köhler*[1]*, A. M. Nijhof*[1,3]
[1]Freie Universitaet Berlin, Institut für Parasitologie und Tropenveterinärmedizin, Berlin, Germany
[2]École Nationale de Médecine Vétérinaire, Laboratoire de Parasitologie, Sidi Thabet, Tunisia
[3]Freie Universitaet Berlin, Veterinary Centre for Resistance Research, Berlin, Germany

Tropical theileriosis (TT) is a protozoan disease caused by *Theileria annulata* that affects cattle in areas where vector ticks (*Hyalomma* spp.) occur. Measures to control TT include vaccination with attenuated *T. annulata* schizonts cultivated *in vitro*. These cell lines are typically cultured in media containing animal sera, mostly Foetal Bovine Serum (FBS). To study tick-pathogen interactions, *Hyalomma* tick colonies are required that are maintained on experimental animals. The development and optimization of methods to cultivate *T. annulata* schizonts *in vitro* in serum-free media (SFM) and to feed ticks artificially without the use of animals would contribute to the 3Rs principle.

Experiments to evaluate the ability of different *T. annulata* strains to be grown, frozen and resuscitated after cryopreservation in SFM were conducted. Life stages of *H. scupense*, *H. dromedarii* and *H. excavatum* were fed *in vitro* following methods previously described for *Ixodes ricinus*.

Freshly isolated *T. annulata*-infected cells could be propagated, frozen and thawed in SFM (ISF-1 medium), but once cells were adapted to cultivation in the presence of FBS or resuscitated from frozen storage, cells propagated in SFM did not perform as well as cells grown in RPMI medium supplemented with FBS. *Hyalomma excavatum* adults fed in vitro, but with low engorgement rates: only two out of 40 ticks (5%) fed to repletion. A high attachment rate was observed for *Hy. scupense* adults (38 out of 40 ticks, 95%), but only five ticks (12.5%) partially engorged and detached (12.5%). A similar trend was observed for *Hy. scupense* larvae, with attachment rates varying from 67.5% (77/114 ticks) to 86.8% (106/122), but only 13 larvae successfully molted to the nymphal stage. For *H. dromedarii*, nymphs could be fed successfully *in vitro* with attachment and engorgement rates of (129/143) 90.2% and 47.6% (68/143), respectively. Further optimization of the artificial feeding of all *Hyalomma* life stages will be required.

O 009

Variation in *Ixodes ricinus* density and pathogen prevalence within forest stands driven by vegetation and local host abundance

M. Van Gestel[1,2], *K. Verheyen*[2], *D. Heylen*[1,3], *H. Sprong*[4], *M. Fonville*[4], *E. Matthysen*[1]
[1]University of Antwerp, Department of Biology, Wilrijk, Belgium
[2]Ghent University, Department of Environment, Gontrode, Belgium
[3]Institute of Tropical Medicine, Department of Biomedical Sciences, Antwerp, Belgium
[4]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands

Spatial variation in density and pathogen prevalence in *Ixodes ricinus* has been studied for decades, identifying vegetation characteristics and host presence as the main drivers of variation between greenspaces. Contrarily, within-greenspace variation is understudied, although its understanding could contribute strongly to reducing tick bite risk. We studied variation in density of nymphs (DON) and nymphal infection prevalence (NIP) within and between forest stands of public greenspaces. Since we expect sharp contrasts in the drivers of DON and NIP near recreational infrastructure such as trails or benches, we selected forest stands accommodating such infrastructure. Drag sampling was combined with camera trapping of potential hosts, vegetation surveys and screening for *Borrelia burgdorferi* s.l., *Rickettsia helvetica*, *Neoehrlichia mikurensis*, *Anaplasma phagocytophilum* and *Borrelia miyamotoi*. Initial models suggested an infrastructure and vegetation effect on the time specific host species spend at given locations, which may subsequently affect the distribution of (infected) ticks. Therefore, GLMMs were drafted to study variation in DON and NIP, including an infrastructure factor and either local host abundance or vegetation characteristics as explanatory variables. In both models DON was lower near trails and benches, compared to nearby forest interior. DON was also higher wherever herb- and canopy-layer vegetation were more developed. We observed trends for positive associations of DON with rodent and songbird abundance, and a negative association with pigeon abundance. The NIP of different pathogens was affected by different drivers, as demonstrated by two genospecies of *B. burgdorferi*: prevalence of *B. afzelii* correlated with infrastructure and vegetation characteristics, whereas that of *B. garinii* did not, but increased with the local abundance of songbirds. Our results support amplification and dilution effects on both a within- and between forest stand level.

O 010

Metagenomic profiling of viruses identified in tick populations collected in Laos

A. Bennouna[1]*, K. Vongphayloth*[2]*, S. M. Ali*[1]*, J. M. Fiorenzano*[3]*, N. Phommavanh*[2]*, K. Lakeomany*[2]
D. Chrétien[1]*, T. Bigot*[1]*, S. Temmam*[1]*, A. G. Letizia*[3]*, P. T. Brey*[2]*, M. Eloit*[1,4]
[1]Institut Pasteur, Pathogen Discovery Laboratory, Paris, France
[2]Institut Pasteur du Laos, Laboratory of Vector-Borne Diseases, Vientiane, Lao People's Democratic Republic
[3]U.S. Naval Medical Research Unit-2-Asia, Singapore, Singapore
[4]Alfort National Veterinary School, Maisons-Alfort, France

Introduction: Ticks are blood-sucking vectors of several human and animal pathogens that can pose a significant public health burden. Next generation sequencing (NGS) allows for the identification of known viral threats including tick-borne encephalitis virus, Crimean-Congo hemorrhagic fever viruses, and thrombocytopenia-causing phlebovirus as well as novel pathogen detection. In Laos, the full range of tick-borne viruses is unknown.

Objectives: The aim of this study was to identify viruses associated with tick species circulating in North Laos, for a comprehensive understanding of the potential risk of emergence in animals and humans living in this area.

Materials and methods: We collected ticks from three rural locations in Northern Laos between 2019 and 2022. Specimens were mainly collected from cows and sometimes from the environment (tick drags).

Total nucleic acids were extracted and NGS library was validated and sequenced using an Illumina technology in a paired-end format. Pathogen identification was then carried out using a dedicated pipeline of viruses, followed by phylogenetic analysis.

Results: A total of 506 minipools from 2,361 ticks, including *Rhipicephalus* (n= 2,016), *Haemaphysalis* (n= 220), *Amblyomma* (n= 59), *Dermacentor* (n= 26) and *Ixodes* (n= 40), were pooled in 58 NGS libraries.

Our analysis showed that the total tick virome is dominated by RNA viruses, belonging to at least 24 viral families including *Flaviviridae*, *Chuviridae, Rhabdoviridae*, *Phenuiviridae* etc., which are known to infect vertebrates, invertebrates, plants, fungi, and algae.

Conclusion: Further analysis is ongoing to define the typology of viruses that could spill over from infected ticks to vertebrates/humans, define bottlenecks that govern arboviral emergence and inform public health policy to prevent infection.

O 011

Molecular and MALDI-TOF MS characterisation of *Hyalomma aegyptium* ticks collected from turtles and their associated microorganisms in Algeria

H. Benyahia[1]
[1]Université Aix-Marseille France, Médecine, Marseille 10e Arrondissement, France

The identification of ticks and their associated pathogens is important for knowledge on tick-borne diseases. The objective of this study was to use morphological, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and/or molecular biology tools to identify ticks collected from turtles in north-eastern Algeria, as well as to investigate the microorganisms associated with these ticks. A total of 471 adult ticks were collected and identified morphologically as *Hyalomma aegyptium*, of which 248 (52.7%) were female and 223 (47.3%) were male. amongst them, 230 specimens were randomly selected for molecular and MALDI-TOF MS analysis. Molecular biology confirmed that our ticks were *Hy. aegyptium*. MALDI-TOF MS analysis revealed that 100% of the spectra were of excellent quality. Four spectra were selected to update our own database MALDI-TOF MS arthropod. The blind test of the 226 remaining spectra showed that all ticks were correctly identified, with scores ranging from 1.774 to 2.655 with a mean of 2.271 ± 0.16 of which, 223 (98.6%) had log score value (LSV)$>$1.8. Molecular biology screening showed that the ticks carried the DNA of *Borrelia turcica, Rickettsia africae, Rickettsia aeschlimannii, Rickettsia sibirica mongolitimonae* and with the Anaplasmataceae were close to a potentially new, undescribed *Ehrlichia* sp. This study confirms that MALDI-TOF MS is a reliable tool for the identification of ticks and that ticks collected from turtles in Algeria are carriers of several species of microorganisms which may be responsible for diseases in humans and animals.

O 012

Linking *Borrelia burgdorferi* peptidoglycan and Lyme arthritis – Correlation or causation?

M. McClune[1,2,3], *J. Dressler*[1,2], *M. Davis*[1,2], *T. DeHart*[1,2], *J. Tupik*[3,4,5], *B. Jutras*[1,2,3,6,7]
[1]Virginia Tech, Biochemistry, Blacksburg, United States
[2]Fralin Life Science Institute, Blacksburg, United States
[3]Center for Emerging, Zoonotic, and Arthropod-borne Pathogens (CeZAP), Blacksburg, United States
[4]Virginia-Maryland College of Veterinary Medicine, Blacksburg, United States
[5]Dept of Biomedical and Veterinary Medicine, Blacksburg, United States
[6]Molecular and Cellular Biology, Blacksburg, United States
[7]Translational Biology, Medicine, and Health, Blacksburg, United States

Lyme Borreliosis (LB) continues to increase in the United States (US) and Europe. *B. burgdorferi* sensu stricto (*B. burgdorferi*), the US predominant strain, can cause debilitating symptoms like carditis, neuroborreliosis, and arthritis. Lyme arthritis (LA) is the most common late-stage symptom of LB affecting ~50,000 people annually in the US. *B. burgdorferi* lacks virulence factors common to pathogenic bacteria. To better understand these manifestations, our studies have focused on *B. burgdorferi*'s peptidoglycan (PG). PG is structurally important to bacteria and typically recycled each generation. *B. burgdorferi* lack the pathways required to recycle PG, releasing 45% of their total PG per generation. Recent work uncovered differences within the PG regarding amino acid incorporation and glycan organization. *B. burgdorferi* PG is proinflammatory, and even persists in LA patient synovial fluid post infection. By intravenously injecting *B. burgdorferi* PG we can mimic LA in mice. This led us to question how *B. burgdorferi* PG persists *in vivo* and its subcellular effects. We developed a real-time, *in vivo* tracking system to study PG biodistribution in live mice. Comparative analyses of bacterial PG uncovered that, while typical PG structures clear within 48 hours, *B. burgdorferi* PG has a distinct biodistribution pattern, lingering in certain tissues for weeks. Since *B. burgdorferi* PG is released, unique, and has long retention times, we performed comparative transcriptomic analyses. Human PBMCs were stimulated with different bacterial PGs and after just 12 hours, *B. burgdorferi* PG altered the transcription of multiple metabolic pathways. Interestingly, our data indicate the PG structures of *B. burgdorferi*, *B. afzelii,* and *B. garinii* are distinct, possibly contributing to the diverse clinical presentations associated with each bacterium. Further studies on the involvement of PG in LB symptoms will help guide targeted therapeutic development to alleviate these manifestations.

O 013

Comparison of neutralizing activity of vaccine-induced TBEV antibodies against 10 genetically different TBEV strains of the European subtypes

F. Greiner[1], *A. Lindau*[1], *U. Mackenstedt*[1], *G. Dobler*[1,2]
[1]University of Hohenheim, Institute of Biology, Department of Parasitology, Stuttgart, Germany
[2]Bundeswehr Institute of Microbiology, National Consulting Laboratory for TBE, Munich, Germany

Introduction: Vaccination against TBEV has been shown to be highly effective. However, differences in the neutralization activity of antibodies have been observed in vaccinated individuals.
Objectives: The aim of this study was to investigate the neutralizing capacity of vaccine-induced antibodies against 10 different TBE-virus strains after anti-TBEV IgG standardisation.
Material & methods: 36 sera from participants in Baden-Wuerttemberg were obtained and divided into three groups: (1) Encepur (participants who had received Encepur® only), (2) FSME-IMMUN (participants who had received FSME-IMMUN® only), (3) Mix (participants who had received at least three vaccine doses, at least one per brand). All sera exhibited high avidities. The 12 sera from each group were titrated by ELISA (EUROIMMUN, Lübeck, Germany) and adjusted to 250 Vienna Units/ml. Then the adjusted sera were tested by micro-neutralization assays against the 10 TBEV strains using a fix antibody concentration and varying virus concentrations (10^1 virus particles/ml to 10^5 virus particles/ml).
Results: No significant differences were found between the groups. Variations within the groups, related to individual sera, were not associated with factors such as age or vaccination history of the individuals. Neutralizing activity was dependent on the viral strain used or on individual factors of the vaccinated individuals, which could not be eliminated by standardization.
Conclusion; The neutralizing activity of the induced antibodies did not differ based on the vaccines used. However, a vaccination history during which both vaccines were used seems to lead to a more uniform antibody response against the different viral strains than in participants who had received only one brand of vaccine.

This project was funded by Pfizer Pharma GmbH.

O 014

Ticks – world's most dangerous vectors. How disguise and glutinous feeding facilitates immense reproductive success and tick-borne disease transmission

D. Sonenshine[1]
[1]National Institutes of Health, LMVR, NIAID, Rockville, Maryland, United States

Most scientists, students, and medical professionals learn about the details of diverse tick-borne pathogens and the diseases that they cause.

What about the ticks themselves? Little is taught about the biology of the tick vectors. Why are they so successful and so widespread?

In this presentation, I will review how hard ticks (Ixodidae) attach to their hosts and engorge enormous quantities of blood. How do they do it?

First, ixodid ticks secrete cement to bind themselves to their hosts' skin, also secreting compounds to evade detection by their hosts' alarm systems. Next, they engorge slowly, digesting hemoglobin, while synthesizing new cuticle, growing a new body to facilitate sucking maximum amounts of blood. Subsequently, after achieving maximum body enlargement, they suck blood rapidly, and eliminate excess blood meal water and salts. These feeding events are controlled by upregulation of specific neuropeptides and neurotransmitters in an exquisitely synchronized order controlled by the tick's synganglion. In adult females, following mating and insemination with spermatozoa and seminal compounds from their male partners, the rapidly engorging females express new signaling neuroregulatory molecules that trigger the synthesis and secretion of vitellogenin (Vg), the precursor for vitellin (yolk). Vg is taken up by specialized receptors on the developing oocytes which soon grow to maximum size. This is the final goal of the lengthy feeding process. Subsequently, the fully engorged female, often expanded to more than 100 times its pre-feeding weight, drops off the host and shelters in protected microenvironments in the natural habitat. In this shelter, oviposition begins. From this point forward, the immensely swollen female becomes an "egg machine", laying thousands of eggs, all carefully coated with wax to facilitate their survival.

O 015

Dual SIFamide neuropeptide receptors in control of *Ixodes* salivary glands

L. Simo[1], *Y. Park*[2], *M. Vancová*[3,4], *F. Guerrib*[5], *C. Ning*[5], *L. Mateos-Hernandez*[5], *S. Rakotobe*[1]
O. Hajdusek[3], *J. Perner*[3], *J. J. Valdés*[3], *T. Bílý*[3,4]
[1]INRAE, French National Research Institute for Agriculture, Food and Environment, Maisons-Alfort, France
[2]Kansas State University, Entomology Department, Manhattan KS, United States
[3]Biology Centre, Czech Academy of Sciences, Institute of Parasitology, Ceske Budejovice, Czech Republic
[4]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
[5]UMR-BIPAR (ANSES, INRAE, ENVA), Animal Health, Maisons-Alfort, France

Introduction: Salivary glands are vital to tick feeding success and also play a crucial role in tick-borne pathogen transmission. More than a decade ago it has been demonstrated that neuropeptide SIFamide targets the basal regions of saliva-producing type II and III acini via neuropeptidergic axons originating from giant protocerebral neurons in the tick synganglion.

Objectives:

- Molecular characterization and functional expression the *Ixodes* SIFamide receptors
- Immuno-localization of SIFamide and its receptors in *Ixodes* salivary gland acini type II and III

Materials and methods:

- Molecular cloning
- Phylogeny
- Heterologous expression
- Wholemount immunohistochemistry
- Immunogold labelling transmission electron microscopy

Results: We identified two authentic *Ixodes* SIFamide receptors (SIFa_R1 and SIFa_R2) making this tick species the first arthropod in which two different SIFa_Rs have been functionally characterized. We also experimentally proved the high biological affinity of insect SIFamide paralogs to *Ixodes* SIFa_R2, but not SIFa_R1. The immunogold staining, in combination with transmission electron microscopy, revealed the expression of both SIFa_Rs on the basal epithelial cells and the single myoepithelial cell close to the apex of the acinar duct. As both these cell types overlay the arms of the acinar valve, we suggest that SIFamide and its two receptors regulate the release of saliva from the acinus to associated ducts by controlling this structure. In addition, the SIFa_R2, found to be expressed on the basal granular cells in both type II and III acini, likely control their secretory activities.
Conclusion: Our findings indicate that the neuropeptide SIFamide acts via two different receptors that regulate distinct or common cell types in the basal region of type II and III acini in *Ixodes* salivary glands. Here we highlight the structural and functional complexity of peptidergic control of *Ixodes* salivary glands, emphasizing its uniqueness among the arthropod phylum.

O 016

Molecular outsourcing of haem as a parasitic strategy of ticks

T. Hatalova[1], *V. Urbanova*[1], *P. Kopacek*[1], *J. Perner*[1]
[1]Biology Centre, Institute of Parasitology, Ceske Budejovice, Czech Republic

Introduction: Tick midgut cells and tick saliva come in direct contact with host molecules, representing two distinct Tick-Host Interfaces. Ticks have lost most of the genomic coding and, thus, biochemical capacity to synthesize haem de novo. Instead, they are equipped with the acquisition of host haem by a specific uptake of host haemoglobin by tick midgut digest cells.
Objectives: We sought to identify and characterise intracellular binders and transporters of haem in digest cells of the tick midgut.
Materials & methods: Using recombinant proteins, UV-VIS based binding evaluation, systemic RNAi, and microbiological growth assays, we characterised tick Glutathione S-transferase (GST) and Haem-responsive gene (HRG).
Results: Ticks (*Ixodes ricinus*) express in their midgut haem-responsive *gst* transcript in response to blood meal. Its expression does not increase in response to serum meal. Such haem-induced GST was then shown to bind haem in vitro, suggesting a possible mopping up of excessive haem that may appear unbound after haemoglobin hydrolysis, in the tick digest cells. Such released haem gets channelled from the digest vacuole through the HRG transporter. The transporting role was determined in Δhem1 *Saccharomyces cerevisiae* model, i.e. a strain deficient in the genetic coding for the first enzyme of haem biosynthesis. Additionally, RNAi-silencing of HRG rescues ticks from toxic haem analogue (GaPPIX) suggesting the role of haem/porphyrin transporter.
Conclusion: We show that ticks encode proteins implicated in haem mobilization, i.e. converting exogenous haem into endogenous haem by its translocation from digestive vesicles in order to fuel the intracellular haem-driven enzymatic network.

References

PMID: 29526768
PMID: 34465215
PMID: 30686614
PMID: 35346896

O 017

The Toll pathway regulates tick immune response

M. Jalovecka[1], V. Urbanova[1], R. Sima[1], P. Kopacek[1], J. Perner[1], O. Hajdusek[1]
[1]Biology Centre, Institute of Parasitology, České Budějovice, Czech Republic

Introduction: Ticks transmit a variety of pathogens to animals and humans through their bite. Pathogens are recognized by the tick innate immune system, which includes Toll and IMD signaling pathways.
Objective: The objective of this work was to investigate the Toll pathway in the tick *I. ricinus* and to elucidate its role in the transmission of tick-borne pathogens.
Materials & Methods: We have used RNAi-mediated silencing of the Toll pathway components, ultimately leading to modulation (suppression or activation) of the tick immune system. We also used laboratory transmission models for *Borrelia afzelii* and *Babesia microti* to test whether the Toll pathway is involved in pathogen recognition, acquisition, and transmission.
Results: We identified homologs of the insect Toll and IMD pathways in *I. ricinus*. In addition, we have identified and genetically validated the first read-out gene of the tick Toll pathway. We observed that expression of this gene is strongly upregulated by injection of the Gram-negative bacterium *E. coli* and that the Toll pathway regulates infection levels of the apicomplexan protozoan *B. microti*.
Conclusion: Our results show that the tick Toll pathway is fully functional, can recognize extracellular bacteria, and responds to their presence by expressing antimicrobial genes. We have also shown that tick immunity plays a critical role in determining *Babesia* infection rates. These results open the door for further identification of molecules and mechanisms that determine the ability of arthropods to transmit apicomplexan parasites.

O 018

Functional Analysis of Folate Biosynthesis Genes of Rickettsia monacensis str. Humboldt via Complementation Assay

J. Zhong[1], B. Hill[1]
[1]Cal Poly Humboldt, Biological Sciences, Arcata, United States

Nutritive symbiosis between bacteria and ticks is observed across a range of microbes and hosts, however, little characterization on the molecular components responsible for these symbiosis has been done. Previous studies in our lab demonstrated that *Rickettsia monacensis* str. Humboldt (RMSH) can synthesize folate de novo via folA, folC, folE, folKP, and ptpS pathway. In this study expression of RMSH folate genes, within folate gene mutant E. coli K-12 strain BW25113, was used to functionally characterize RMSH folate genes in vivo. RMSH folate genes were subcloned into TransBac vector and transformed into folA, folC, folE or folK knockout mutant E. coli K-12 strain BW25113. Each mutant containing RMSH subclone and pFE604 clone of knocked-out folate gene, was cured of pFE604. Curing of folA mutant was successful using acridine orange and 43.5°C but curing folE and folK mutants was only achieved after supplementing plasmid curing media with folate end-products. Preliminary plasmid curing assay showed curing efficiency of folA mutant at 100%, whereas curing efficiencies for other mutants was 0% to 10%. Functional complementation was assessed by growth phenotype on minimal media with and without IPTG between RMSH folA and E. coli folA as well as RMSH folC and E. coli folC gene pairs. Large and homogeneous wild-type colony growth was observed for both assayed gene pairs on minimal media with IPTG, and lack of growth or pin-point growth without IPTG. This study provides evidence substantiating the in vivo functionality of RMSH folate genes in producing functional gene products for folate biosynthesis.

O 019

What is the origin of the “core” microbiome of ticks?

N. Fernández Ruiz[1]*, S. Pinecki Socias*[2]*, A. Estrada-Peña*[1]*, A. Wu-Chuang*[3]*, A. Maitre*[3]
A. Cabezas-Cruz[3]*, I. De Blas*[4]*, A. Nijhof*[2]
[1]University of Zaragoza, Faculty of Veterinary Medicine. Parasitology Department, Zaragoza, Spain
[2]Freie Universität, Institute of Parasitology and Tropical Veterinary Medicine, Berlin, Germany
[3]ANSES, INRAE Ecole Nationale Vétérinaire d’Alfort, Laboratoire de Santé Animale, Paris, France
[4]Faculty of Veterinary Medicine, Microbiology, Zaragoza, Spain

Question: Studies on the microbiota of ticks have promoted interesting hypotheses about the combined effects of the bacterial community, its functional contributions to the tick’s physiology or the probable competition effects with some tick-borne pathogens. However, basic knowledge on the topic is still missing. The current study aims to elucidate the source(s) of the microbiota in unfed tick larvae, addressing basic questions about the composition of the “core” microbiota and the best ways to obtain decontaminated eggs (i.e. keeping only the internal bacteria, free of external contaminations).
Methods: We used engorged females of *Rhipicephalus australis* ticks divided into four groups of treatments, based on laboratory quality bleach washes and/or ultraviolet light (UV) treatment. Bleach wash was applied on both engorged ticks and eggs, while the UV treatment was only applied to the females. Then we used the next sequencing techniques to obtain the microbiota data from the different groups and subjected them to statistical analyses.
Results: Results point to the ovary as the major contributor to the microbiota of unfed tick larvae, with a small contribution from Gené’s organ. The contribution of male sperm could not be discarded but has been not addressed in the current protocols. Both bleach and UV had no significant effects on the reproductive efficiency of engorged females and the hatching rates of eggs. However, the protocols of decontamination produced striking results. Data suggest that bleach washes seem to disturb the internal tick microbiota (like *Borrelia* or *Coxiella*) only in the treated females with bleach, indicating the possibility of the entry of bleach into the tick affecting its internal microbiota.
Conclusions: Further studies are necessary to establish the effect of decontaminating treatments on ticks and their eggs in order to establish the “core” microbiota, and the interactions among co-occurring taxa including pathogen-tick interactions.

O 020

TBEV at the blood brain barrier and behind

M. Palus[1,2], *M. Vancova*[1], *V. Pranclova*[1,3], *V. Hönig*[1,2], *E. Kotounova*[1,3], *J. Tyc*[1], *D. Ruzek*[1,2,4]
[1]Biology Centre CAS, Institute of Parasitology, Ceske Budejovice, Czech Republic
[2]Veterinary Research Institute, Department of Virology, Brno, Czech Republic
[3]University of South Bohemia, Faculty of Sciences, Ceske Budejovice, Czech Republic
[4]Masaryk University, Faculty of Science, Brno, Czech Republic

Tick-borne encephalitis virus (TBEV), a member of the genus *Flavivirus* in the family *Flaviviridae*, is the leading flaviviral neuroinfection in Europe and northeast Asia and causes severe disease, often with long-lasting debilitating consequences in surviving patients. Pathology following TBEV infection is the result of viral infection and the inflammatory response associated with blood-brain barrier (BBB) disruption. However, the role of the non-neuronal cells that form the BBB in the development of TBE, particularly in the susceptibility, permissivity, and spread of the virus to the CNS, is not fully understood. We have studied the pathology that occurs after TBEV infection *in vivo* in mouse models but also in the individual cellular components of the neurovascular unit (NVU), ie, primary human brain microvascular endothelial cells, pericytes, astrocytes, and neurons. In addition to NVU cells, we also tested human microglial cells. All of these cell types are susceptible to TBEV infection, but the replication kinetics of the virus and the percentage of virus-infected cells in culture are different. Our monoculture transwell model demonstrated that virus can pass through the BBB via the transcellular pathway without compromising BBB integrity. Correlative immunofluorescence and scanning electron microscopy confirmed the presence of an unaltered and well-organized tight junctions between TBEV-infected or infected and uninfected cells. Infection of astrocytes and pericytes led to their activation and induced the expression of proinflammatory cytokines. The 3D topographic organization of membranous whorls and autophagic vacuoles in TBEV-infected human cells was mapped in parallel with serial block-face scanning electron microscopy from the *in vivo* mouse model.
The results provide new insights into the interactions between TBEV and cellular components of the neurovascular unit that may contribute to TBEV-induced neurotoxicity and BBB disruption.

O 021

Transmission of the tick-borne encephalitis virus by hybrids of *Ixodes ricinus* and *Ixodes persulcatus* ticks

O. Belova[1], *A. Polienko*[1], *A. Averianova*[1], *G. Karganova*[1]
[1]FSASI “Chumakov FSC R&D IBP RAS” (Institute of Poliomyelitis), Laboratory of Biology of arboviruses, Moscow, Russian Federation

The evolution of the tick-borne encephalitis virus (TBEV), the most important human arboviral pathogen in Europe and Asia, has led to the formation of three stable subtypes, connected with certain vector species: the European subtype, associated with *Ixodes ricinus*, and the Siberian and Far-Eastern subtypes, associated with *Ixodes persulcatus* ticks. Distribution ranges of these tick species overlap and form large sympatric areas in the East European Plain and Baltic countries. It has previously been shown that crossing between *I. ricinus* and *I. persulcatus* is possible, with the appearance of sterile hybrids. Hybridization of ticks can affect not only the spread of ticks but also the properties of natural foci of arbovirus infections, in particular TBEV. In the present study, we analyzed the effectiveness of virus transmission from infected mice to larvae and nymphs and trans-stadial transmission (from larvae to nymph and adult) in *I. ricinus*, *I. persulcatus,* and hybrids.

For this purpose, we bred a hybrid generation from the crossing of *I. persulcatus* females and *I. ricinus* males, and we used the Siberian and European subtypes of TBEV. We showed that after feeding on infected mice, virus prevalence in engorged ticks decreased over time, and after molting, the opposite was true. In hybrids we observed the highest acquisition effectiveness and RNA copy numbers during Siberian subtype transmission. The efficiency of trans-stadial transmission of both TBEV subtypes was similar in hybrids and parental species. After the second trans-stadial TBEV transmission, a significant increase in ticks' infection rates was observed only in specific subtype-tick combination. Our data demonstrate the possible features of TBEV circulation in the *I. ricinus* and *I. persulcatus* sympatry area. This information will be extremely useful for understanding the tick–virus interactions under the conditions of the expanding sympatric zone of the main TBEV vectors and the switching of the vector.

O 022

Complete genome sequencing of tick-borne encephalitis virus reveals potential mutations in viral genome associated with disease course

S. Zakotnik[1], *N. Knap*[1], *A. Suljič*[1], *D. Vlaj*[1], *P. Bogovič*[2], *F. Strle*[2], *M. Korva*[1], *T. Avšič Županc*[1]
[1]University of Ljubljana Medicine Faculty, Institute of Microbiology and Immunology, Ljubljana, Slovenia
[2]University Medical Centre Ljubljana, Department of Infectious Diseases, Ljubljana, Slovenia

Introduction: Tick-borne encephalitis (TBE) is the most important viral infection of the human central nervous system in Slovenia and is caused by the TBE virus (TBEV). TBEV infections vary, from asymptomatic infection to CNS inflammation. The factors that influence disease progression and outcome are largely unknown. Infection is usually diagnosed in the post-viremic phase, therefore, clinical samples with TBEV RNA are extremely rare.
Objective: The objective of this study was to use previously developed protocols for direct sequencing of the TBEV genome from clinical samples for studies of viral genetic determinants that influence disease severity.
Materials & Methods: 94 blood samples were collected from patients in the viremic phase of the disease. The courses were mild to moderate in 44 cases (9x abortive form, 35x meningitis) and severe in 50 cases (44x meningoencephalitis, 6x meningoencephalomyelitis). Viral RNA was isolated, amplified with virus-specific primers, and sequenced by NGS. Data were analyzed, and variant calling and consensus viral sequences were generated.
Results: We successfully generated 94 near-complete TBEV genome sequences (average 8% or less N) directly from clinical samples. Analysis of major mutations in TBEV genomes showed that more synonymous mutations in the coding sequence for the E protein are associated with less severe disease progression ($p=0.039$). Quasi-species (QS) analysis showed that more synonymous QS in the coding sequence for the NS1 protein are associated with less severe disease progression ($p=0.0363$). But more non-synonymous

QS in the same region show a trend toward more severe courses. Analysis of QS in the coding sequence for the NS5 protein shows a trend toward less severe course in samples with more synonymous QS. Conclusion: Sequencing of the TBEV genome directly from clinical samples has shown that viral determinants are associated with TBE disease course and points to the genomic regions containing critical mutations.

O 023

TBE virus sero-prevalence in TBE highly endemic areas

G. Dobler[1], K. Euringer[1,2], P. Girl[1], J. Peilstöcker[3], M. Schmidt[3], J. Borde[2,4]
[1]Institut für Mikrobiologie der Bundeswehr, München, Germany
[2]University Medical Center Freiburg, Division of Infectious Diseases, Freiburg i. Br, Germany
[3]DRK Blutspendedienst Baden-Württemberg – Hessen gGmbH, Frankfurt a. M., Germany
[4]Praxis Prof. Borde & Kollegen, Oberkirch, Germany

Tick-borne encephalitis (TBE) is the most important tick-borne viral disease in Europeand Asia. Since the 1980s, when the TBE vaccines have been introduced,seroprevalence data are difficult to obtain, as vaccine- and infection-induced antibodies could not be distinguished. Therefore, the true incidence of TBE in the population is unknown. Information dates back to the 1950. and 1960s.

Using a new anti TBE-NS1-IgG assay the prevalence of TBE infection versus TBE vaccination in a highly endemic region population in southwestern Germany was studied. 2,220 sera from blood donors from the district Ortenaukreis were tested for antiTBE-IgG. IgG-positive sera were further differentiated using the antiTBE-NS1-IgG. antiTBE-IgG positive and antiTBE-NS1-IgG negative sera were further confirmed by a micro-neutralization assay.

A total of 1,257/2,220 (57%) was tested positive for anti-TBEV-IgG. 125/1,257 antiTBE-IgG sere were found positive for infection-induced TBEV-NS1-IgG. This means that about 5.6% of the population were infected with TBE virus and regarding the non-vaccinated portion of population 11.8% had been infected during the last 20 years. Similar results dating back from 1986 show that the prevalence of TBE virus infection increased more than 14-fold.
The data show for the first time for more than 40 years that TBE virus infection is still relevant in highly endemic areas and that it poses a realistic and much higher risk than estimated, only from the officially reported human cases. The vaccination rate of 51% is much higher as reported by public data. These data also imply that the infections risk is much higher than estimated so far.

O 024

Ecology of Powassan virus transmission in North America

S. Thangamani[1], *C. Hart*[1], *C. Vogels*[2], *N. Grubaugh*[2]
[1]SUNY Upstate Medical University, Microbiology and Immunology, Syracuse, United States
[2]Yale University, New Haven, United States

Powassan virus (POWV) is a member of the tick-borne encephalitis virus (TBEV) serogroup found in Canada and the United States. It causes encephalitis, including fatal neuroinvasive disease in approximately 15% of cases, and long-lasting neurological sequelae have been documented in 50% of survivors. POWV has recently re-emerged, as evidenced by the increased number and expanding geographic range of confirmed cases. It is transmitted to small- and medium-sized mammals by *Ixodes scapularis*, *Ixodes cookei*, and several other *Ixodes* tick species. Humans become infected with POWV during spillover transmission from the natural transmission cycles. POWV was recognized as a human pathogen in 1958 when a young boy died of severe encephalitis in Powassan, Ontario, Canada. Two distinct genetic lineages of POWV are now recognized: POWV (lineage I) and deer tick virus (lineage II). Since the index case in 1958, over 170 human cases of POWV have been reported in the United States, with an apparent rise in disease incidence in the past 17 years. This recent increase in cases may represent a true emergence of POWV in regions where the tick vector species are prevalent, or it could represent an increase in POWV surveillance and diagnosis. We are tracking the emergence of the Powassan virus in the United States through our community-engaged tick surveillance program (www.nyticks.org). Data collected from this program has allowed us to identify hot spots for Powassan virus transmission. We are currently conducting one health surveillance in the hot spots to better understand ecological and environmental factors influencing the transmission and perpetuation of the Powassan virus in the hot spots. Results generated from this study will allow us to better understand why the Powassan virus emergence is highly restricted to micro-foci niches.

P 001

Spotted fever group rickettsiae in ticks and small mammals in grassland and forest habitats in Central Germany

C. Arz[1], *A. Obiegala*[1], *N. Król*[1], *C. Imholt*[2], *K. Jeske*[3], *R. G. Ulrich*[3], *J. Jacob*[2], *Z. Rentería-Solís*[4]
M. Pfeffer[1]
[1]University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany
[2]Julius Kühn-Institute, Institute for Epidemiology and Pathogen Diagnostics, Münster, Germany
[3]Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases, Greifswald – Insel Riems, Germany
[4]University of Leipzig, Institute for Parasitology, Centre for Infectious Diseases, Leipzig, Germany

Rickettsiae of the spotted fever group (SFG) are zoonotic tick-borne pathogens. Small mammals are important hosts for immature life stages of two of the most common tick species in Europe, *Ixodes ricinus* and *Dermacentor reticulatus* and of associated SFG rickettsiae.

This study aims to investigate the influence of environmental and individual factors on the prevalence of SFG rickettsiae in ticks and small mammals in different small-scale habitats in Central Germany for the first time.

In total, ten species of small mammals and two species of ticks were collected in grasslands and forests in Hainich Dün region. After species identification, 1098 ticks and ear snips of 1167 small mammals were screened for *Rickettsia* spp. DNA by qPCR targeting the *gltA* gene. Positive samples were additionally tested by a conventional PCR targeting the *ompB* gene and amplicons were subsequently sequenced.

The overall *Rickettsia* prevalence in ticks was 11.4% and 9.9% in small mammals. *Rickettsia* DNA was detected in 8 out of 10 small mammal species. Prevalence in small mammals was higher in forests (14.1%) than in grasslands (3.6%). Prevalence was highest in *Apodemus* in forests (14.8%) and lowest in *Microtus* (6.6%) inhabiting grassland. Habitat did not influence infection probability of ticks. Prevalence was higher in *D. reticulatus* (46.3%) than in *I. ricinus* ticks (8.6%). Adult ticks were significantly more often infected than nymphs. All sequenced rickettsiae from *I. ricinus* ticks were *R. helvetica* and all from *D. reticulatus* were *R. raoultii*.

Unlike adults, questing *I. ricinus* nymphs have had only one blood meal, which might explain their lower prevalence. Interestingly, habitat type did not influence the prevalence of *Rickettsia* in ticks but in small mammals. A possible explanation may be the high prevalence in small mammals of the genus *Apodemus*, which are more abundant in forests.

P 002

Tick paralysis due to *Ixodes gibbosus*? – Enigmatic cases in domestic mammals from Cyprus

A. Diakou[1]*, A. Foucault Simonin*[2]*, G. Antoniou*[3]*, J. Valdes*[4]*, A. Cabezas-Cruz*[2]*, G. Földvári*[5]
[1]Aristotle University of Thessaloniki, School of Veterinary Medicine, Laboratory of Parasitology and Parasitic Diseases, Thessaloniki, Greece
[2]ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, France
[3]Privet Veterinary Practice, Polis Chrysochous, Cyprus
[4]Biology Centre of the Czech Academy of Sciences, Institute of Parasitology, Branisovska, Czech Republic

[5]Centre for Eco-Epidemiology, National Laboratory for Health Security, Budapest, Hungary

Tick paralysis is a potentially fatal condition, caused by the bite of over 60 different tick species. This condition is induced by diverse toxins, produced and secreted by the salivary glands of female hard ticks and larval soft ticks. The toxins reduce neurotransmission in hosts, leading to flaccid paralysis. Tick paralysis occurs globally with some regions of the world (e.g., USA, Canada and Australia) showing higher prevalence. This survey reports on clinical and epidemiological observations of tick paralysis cases in animals from Cyprus, and preliminary molecular investigations on the nature of responsible toxins.

Local veterinarians have reported typical tick paralysis cases occurring in goats, sheep, dogs and cats, confirmed by anamnesis, blood and biochemical workout results within normal range, clinical examination revealing the presence of ticks predominantly on the head and around the neck, and recovery in 4-24 hours by tick removal alone. Most cases occur in a confined area of Cyprus, between September and March, with a 3- and a 7-year periodicity, differentiated by clinical severity and consequent animal losses. These cyclic occurrence patterns may be attributed to external factors, self-oscillations of the disease system, or joint action of such mechanisms, as described in other tick-borne diseases. Ticks collected from a recent case in a goat were morphologically and molecularly identified as *Ixodes gibbosus*. The tick *Ixodes ricinus* is phylogenetically related to *I. gibbosus*. Bioinformatics revealed a putative *I. ricinus* Kunitz toxin ~35% similar to a spider and scorpion homolog. However, preliminary attempts failed to detect any *I. gibbosus* mRNA homologs. Efforts should be made to characterise specific toxins involved in cases of tick paralysis and towards developing a vaccine that would prevent significant small ruminant losses in free-ranging farming, a common management practice in many areas of the world.

P 003

Using multiple ecological niche modelling approaches to determine the geographical distribution of *Ixodes ricinus* and *Dermacentor reticulatus* in Europe.

M. Noll[1], *B. Makepeace*[1], *R. Wall*[2], *G. Ng*[3], *H. Vineer*[1]
[1]University of Liverpool, Neston, United Kingdom
[2]University of Bristol, Bristol, United Kingdom
[3]Merck & Co., Inc., London, United Kingdom

Ticks transmit a wider variety of pathogens to humans, companion animals and wildlife than any other arthropod in Europe. Two of the most important species include *Ixodes ricinus* and *Dermacentor reticulatus.* As important vectors of disease it is imperative that we have robust estimates of their current distribution across Europe in order to shape risk assessments and intervention strategies, as well as create a baseline to which past and future changes in their distribution can be compared. To achieve this, a series of ecological niche models, using different combinations of input data were constructed and assessed with the aim of establishing which most appropriately represents each species. Species occurrence records obtained from systematic literature searches and GBIF were thinned to different degrees to determine which best removed sampling spatial bias. Four sets of environmental variables were assessed, the commonly utilised BioClim data, its derivative WorldClim data, Terraclimate data and satellite derived variables. Different spatial extents of these variables were also examined, as no consensus on the most appropriate has been established. All combinations of occurrence data, environmental variables and spatial extents were run on three modelling frameworks, maximum entropy (MaxEnt), generalised additive model and random forests. The resultant geospatial species distribution predictions were then compared in combination with statistical evaluations, to determine which combination most appropriately described the current distribution of each species. It was concluded that no one set of model parameters best described both species, and hence there is not one “best practice”.

P 004

Comparison of erythema migrans in patients with or without neurologic involvement

K. Ogrinc[1], S. Lotrič-Furlan[1], V. Maraspin[1], P. Bogovič[1], T. Rojko[1], T. Cerar Kišek[2], E. Ružić-Sabljić[2] G. P. Wormser[3], K. Strle[4], F. Strle[1]
[1]University Medical Centre Ljubljana, Department of Infectious Diseases, Ljubljana, Slovenia
[2]Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia
[3]New York Medical College, Division of Infectious Diseases, Valhalla, New York, United States
[4]Wadsworth Center, NYS Department of Health, Division of Infectious Diseases, Albany, NY, United States

Introduction. Previous reports showed that patients with Bannwarth syndrome (BS) have longer duration of erythema migrans (EM) at diagnosis compared to those without neurologic symptoms. The reasons for this difference are not known.

Objectives. The aim of our study was to characterize EM in patients with BS, in other forms of Lyme neuroborreliosis (LNB), and in those without neurologic involvement.

Methods. We compared the characteristics of EM in 114 patients with BS, 325 patients with suspected and 69 with confirmed LNB without radicular pain and 12,384 patients without neurologic symptoms. All patients were ≥18 years old and met the European diagnostic criteria for EM.

Results. Compared to EM in patients without neurological symptoms, patients with BS were older, had a significantly higher frequency of EM on head/neck and trunk, similar frequency on arms, but lower frequency on legs, longer duration of EM prior to diagnosis, and larger diameter of EM. Moreover, patients with BS had almost exclusively *B. garinii* isolated from the skin. When compared to EM patients with *B. garinii* infection but without neurological involvement, those with BS reported lower frequency of local and constitutional symptoms other than radicular pain, possibly because the intense radicular pain “masked” other minor local or constitutional symptoms. Similar findings were observed when comparing EM in patients with BS to those with suspected or confirmed LNB without radicular pain, suggesting that these distinct EM characteristics are unique to patients with BS.

Conclusions. The EM skin lesion in patients who develop BS has several distinct clinical characteristics to the EM lesion in patients without neurologic involvement or those with other forms of LNB.

P 005

Prevalence of *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and *Babesia* spp. in questing *Ixodes ricinus* across recreational areas in England and Wales.

S. Gandy[1], *K. Hansford*[1,2], *B. Cull*[1], *L. McGinley*[1], *E. Gillingham*[1,2], *A. Vaux*[1], *R. Smith*[3], *A. Semper*[4] *T. Brooks*[4], *M. Fonville*[5], *H. Sprong*[5], *P. Phipps*[6], *N. Johnson*[6,7], *J. Medlock*[1,8]
[1]UK Health Security Agency, Medical Entomology and Zoonoses Ecology, Salisbury, United Kingdom
[2]UK Health Security Agency, Health Protection Research Unit in Environmental Change and Health, Salisbury, United Kingdom
[3]Public Health Wales, Health Protection Division, Cardiff, United Kingdom
[4]UK Health Security Agency, Rare and Imported Pathogen Laboratory, Salisbury, United Kingdom
[5]National Institute for Public Health and the Environment, Centre for Infectious Disease Control, Bilthoven, Netherlands
[6]Animal and Plant Health Agency, Addlestone, United Kingdom
[7]University of Surrey, Faculty of Health and Medicine, Guilford, United Kingdom
[8]National Institute for Health and Care Research, Health Protection Research Unit in Environmental Change and Health, London, United Kingdom

Understanding the variation in pathogens infections rates in *Ixodes ricinus* ticks is important for assessing potential transmission of tick-borne diseases. The aim of this study was to conduct a snapshot survey at recreational areas in England and Wales to understand infection prevalence for three tick-borne transmitted pathogens. *Ixodes ricinus* nymphs were collected each spring at 20 recreational areas across England and Wales between 2014 and 2019. Questing nymphs were individually tested for presence of three pathogens; *Borrelia burgdorferi* s.l. (causing Lyme disease), *Anaplasma phagocytophilum* (causing anaplasmosis) and *Babesia* spp. (causing babesiosis). Regarding *B. burgdorferi* s.l., 4104 nymphs were individually tested. Site-specific *B. burgdorferi* s.l. infection rates in *I. ricinus* nymphs varied from 0% to 9.7% depending on locations, with an average infection rate of 4.0%. Genospecies composition of sequenced samples was 62.5% *B. garinii*, 0.3% *B. valaisiana* and 17.2% *B. afzelii*. *Anaplasma phagocytophilum* was detected in 3.6% of questing nymphs (n=3919 tested), ranging from 0% to 20% depending on the location. Ecotype I accounted for 87% of positive samples and ecotype II for 13%. *Babesia* spp. were detected in 0.4% of ticks (n=3931), ranging from 0% to 2.0%. Northern England and Wales had higher infection rates for *A. phagocytophilum* (4.7% and 12.1%) whilst *B. burgdorferi* s.l. infection rates were higher in southern (5.4%) and northern (2.3%) England. *Borrelia burgdorferi* s.l. infection rates were higher in woodland (5.6%) compared to grassland (2.6%) whilst more ticks were infected with *A. phagocytophilum* in grassland (6.7%) compared to woodland (0.6%). Infection rates for the three studied pathogens varied depending on geographical locations and this study increases the knowledge on the prevalence of causing agents of tick-borne diseases infection in recreational areas across England and Wales.

P 006

Tick-borne pathogens in European bison (*Bison bonasus*) from Lithuania and Poland

A. Paulauskas[1], *D. Černevičienė*[1], *A. Kibiša*[1], *J. Radzijevskaja*[1], *A. Aleksandravičiene*[1], *L. Griciuviene*[1]
I. Lipatova[1], *I. Ražanske*[1], *K. Anusz*[2], *A. Didkowska*[2]
[1]Vytautas Magnus University, Biology, Kaunas, Lithuania
[2]Warsaw University of Life Sciences, Institute of Veterinary Medicine, Warsaw, Poland

Wild ruminants are involved in the transmission of various tick-borne pathogens. The aim of this study was to investigate the presence of tick-borne pathogens in free-ranging and captive European bison from Lithuania and Poland. The tissue samples were collected and screened for *Anaplasma, Babesia,* and *Borrelia* spp. by RT-PCR and nested PCR based on different gene loci. A total of 85 samples of European bison were tested (55 from Lithuania and 30 from Poland). The results of RT-PCR revealed that European bison were infected with *Anaplasma* (40%), *Babesia* (7%), and *Borrelia* (13%) spp. All positive samples were tested further in nested PCRs using a set of genus-specific primers. Positive PCR products of good quality were subjected to sequence analysis. The phylogenetic analysis of the obtained sequences revealed the presence of *Anaplasma phagocytophilum, Babesia divergens, Babesia capreoli,* and *Borrelia burgdorferi* s.l. in tested samples of European bison. This study showed that the European bison may play a role as a natural reservoir for tick-borne pathogens.

P 007

Tick-borne microorganisms detected in small mammals collected in the surroundings of two villages in Styria, Austria

M. Wijnveld[1], *K. Kantner*[1], *M. Ruivo*[1], *A. M. Schötta*[1], *H. Stockinger*[1], *T. Bečvář*[2], *V. Dvořák*[2]
B. Vojtková[2], *J. Sádlová*[2], *E. Kniha*[3], *P. Volf*[2], *J. Walochnik*[3]
[1]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology; Institute for Hygiene and Applied Immunology, Vienna, Austria
[2]Charles University, Department of Parasitology, Prague, Czech Republic
[3]Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria

Tick-borne microbes rely on a complex infection model involving ticks and reservoir animals to sustain in nature. In this study we aimed to get a further insight into the role of small mammals as reservoirs of tick-borne pathogens.
To this end, 87 small mammals (i.e., *Mus musculus*, *Sorex araneus*, *Apodemus flavicollis* and *Myodes glareolus*) were collected near two Austrian villages: Unterpurkla and Hummersdorf. Both villages are in the federal state of Styria.
Samples of the bladder, ear, heart, kidney, liver, lungs, paw and spleen were collected for DNA isolation and subsequent reverse line blot (RLB) analysis. This method allows to screen multiple samples for the presence of DNA of numerous pathogens at once. Briefly, the RLB consists of a membrane that has up to 43 (geno)species specific oligonucleotides bound to it. To screen for pathogens, tissue DNA extractions were subjected to several genus-specific PCRs targeting *Anaplasma*, *Babesia*, *Borrelia*,

Ehrlichia, *Rickettsia* and *Theileria* species. Resulting PCR products were subsequently hybridised to the RLB probes using a miniblotter. Bound PCR products were visualized using chemiluminescence. Of all screened animals, 56% (49/87) tested positive for at least one pathogen. The identified pathogens were *A. phagocytophilum*, *Ba. canis*, *T.* (*Ba.*) *microti*, *B. afzelii*, *B. garinii/bavariensis*, *Cand.* Neoehrlichia mikurensis, *R. helvetica*, *R. raoultii* and *R. slovaca*. Some RLB signals required further sequencing to give a definitive answer. This revealed the presence of a *Hepatozoon* species with an 18S rRNA sequence 99.3% identical to *Hepatozoon ophisauri*, a pathogen previously not found in Austria and normally associated with legless lizards (*Pseudopus apodus*) found from Southern Europe to Central Asia.

This study revealed a surprising variety and high prevalence of tick-borne microbes in various species of collected animals, emphasizing the important role of small mammals as reservoirs of pathogenic agents in nature.

P 008

Characterization of the primitive immune system of *Borrelia afzelii* and *Borrelia garinii*

M. Ruivo[1], *N. Kovács*[1], *M. Wijnveld*[1]
[1]Institute for Hygiene and Applied Immunology – Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Wien, Austria

One of the roles of the restriction modification system (RMS) is to protect bacteria against the introduction of foreign DNA into the cells. This system comprises two types of proteins: restriction endonucleases and DNA methyltransferases. Both systems provide a defence against for example bacteriophages.

Lyme borreliosis is one of the most prevalent tick-borne diseases in Europe and the principal causative agents of this disease in humans in Europe are the members of the *Borrelia burgdorferi* sensu lato species complex (e.g., *B. afzelii*, *B. garinii* and in lesser degree *B. burgdorferi* sensu stricto). Previous studies identified and characterized two RMS genes in *B. burgdorferi* sensu stricto. Namely, *bbe02* and *bbq67*, both with methyltransferase activity. Other species of the complex have not been studied so far and in our study we focus on *B. afzelii* and *B. garinii*. In Europe, B. afzelii is the main cause for skin manifestations such as erythema migrans.

Our aim was to identify and characterize the restriction modification system in *B. afzelii* and *B. garinii*. We analysed the genome of B. afzelii in search of possible RMS genes and selected several candidates. All selected genes were cloned into a plasmid and subsequently used to transform a dam-deficient *Escherichia coli* strain. After inducing the protein transcription, plasmids were reisolated and analysed with methylation-sensitive restriction enzymes. We were able to successfully proof the methyltransferase activity of all genes.

By better understanding the borrelial defence system against foreign DNA, it might be possible to develop a targeted strategy to cure Lyme borreliosis based on bacteriophages in the future. This would reduce the need for antibiotics that not only affect the intended bacterial infection but also beneficial bacteria within the body.

P 009

Infestations of *Rhipicephalus* ticks – evaluations of (unique) case reports covering the last 4 years

K. Fachet[1], A. Lindau[1], U. Mackenstedt[1]
[1]University of Hohenheim, Institute of Biology, Department of Parasitology, Stuttgart, Germany

Introduction: *Rhipicephalus* ticks, especially the brown dog tick (*Rhipicephalus sanguineus* s.l.), are cosmopolitan hard ticks that are repeatedly introduced by dogs into non-endemic areas, such as Germany. *Rh. sanguineus* s.l. is endemic in the Mediterranean area and mainly introduced to Germany by travellers with dogs and dog imports. As the brown dog tick can survive and reproduce indoors, it becomes a burden to dog owners and dogs in their homes.
Objectives: The import routes to Germany and the impact of *Rhipicephalus* ticks in households were investigated in a citizen-science study over a period of three years (2019-2021) and further cases were notified in 2022. Reported cases were classified to make comparisons.
Material & Methods: Cases with infestations of *Rhipicephalus* ticks (mainly in Germany) were documented and evaluated. Case evaluation was based according to the following criteria: Infestation intensity (no. of ticks), acaricides used prior to introduction, import route (vacation or animal rescue), place of origin of the ticks, and utilization of professional help.
Results: The evaluation of the cases showed that both vacations and dog imports are responsible for the introduction of the ticks. Common to all cases is that no acaricides or inadequate acaricides were used. Of the total 38 cases reported, 8 stand out with a special story, or arise due to external influences and could not be resolved completely.
Conclusion: The brown dog tick is becoming more and more in the focus of dog owners. Not only because of the vector competence for various pathogens, but also because of the burden of infestation in homes. Therefore, case reports may provide new information about the introduction of this tick species into non endemic areas.

P 010

Immune-related genes in the fat body of tick *Ixodes ricinus*

V. Urbanová[1], L. Martins[2], E. Kalinova[3], S. Lu[4], J. Ribeiro[4], O. Hajdusek[1], J. Perner[1], P. Kopacek[1]
[1]Biology Centre, CAS, Institute of Parasitology, Ceske Budejovice, Czech Republic
[2]NIH/NIAID, Rocky Mountain Laboratories, Hamilton, Montana, United States
[3]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
[4]NIH/NIAID, Bethesda, United States

Ticks are the vectors of a variety of pathogens. Transmission of pathogens depends on their ability to evade or inhibit the tick´s immune response. A critical role in invertebrate immunity is played by antimicrobial peptides produced primarily by the fat body or hemocytes. The fat body of the tick is a diffuse organ connected to the tracheal trunks (FB / TR) or surrounding other internal organs. Despite its importance, the physiological function of the tick fat body is still relatively unexplored.

To fill this knowledge gap, we performed an RNAseq analysis of the FB / TR complex (NCBI Bioproject PRJNA748353) and processed the data obtained.
The FB / TR complex was dissected from partially fed adult *Ixodes ricinus* females and total RNA samples were submitted for Illumina RNA library construction and sequencing (North Carolina State GSL, Raleigh, NC, USA). Quantitative RT-PCR was used to assess the expression of the selected immunity genes in the biological samples.
In total, we identified about 45,000 transcripts, of which about 1% of the contigs were classified as immunity-related genes. Approximately 20 abundantly expressed transcripts representing different classes of immune molecules were selected and analyzed for their response to microbial immune challenges at different time points. The highest immune response was found after bacterial infection with Gram (-) *Escherichia coli* in 13 of 20 selected genes. Only 4 genes were significantly upregulated after infection with *Micrococcus luteus* Gram (+) bacteria and 4 genes after infection with the yeast *Candida albicans*. The expression of 7 immune genes was not affected by any microbe.
These data may help to better understand immune processes and their regulation in ticks. The role of immune genes in the interaction between ticks and transmitted pathogens will be described in more detail and their role in pathogen transmission will be investigated.
This work was supported by GACR (No. *20-05736S* to P.K.)

P 011

The Tick Cell Biobank – new European tick cell lines

C. Hartley[1]*, J. J. Khoo*[1]*, A. Paloma*[2]*, A. Darby*[1]*, B. Makepeace*[1]*, L. Bell-Sakyi*[1]
[1]University of Liverpool, Infection Biology, Liverpool, United Kingdom
[2]Hospital Universitario San Pedro-CIBIR, Centre of Rickettsiosis and Arthropod-Borne Diseases, La Rioja, Spain

Introduction: Tick cell lines are important tools for research on ticks and the pathogens they transmit. The Tick Cell Biobank (TCB) is the world's only dedicated culture collection for cell lines derived from ticks and other arthropods. Part of the remit of the TCB is to generate novel cell lines from ticks and other arthropods, that are then made available as a resource for the international research community. Until recently, European tick species were underrepresented in the TCB"s portfolio.
Objective: We aimed to increase the number of available cell lines derived from European tick species.
Materials and Methods: Eggs laid by engorged female ticks sourced from Spain and Germany were processed to yield primary cell cultures. These were maintained for up to eight years before subculture series were initiated. Species origin of resultant cell lines was confirmed by sequencing of 16S rRNA gene fragments amplified by PCR. Absence of contaminating microorganisms was confirmed by pan-bacterial PCR.
Results: New cell lines have been generated from *Argas reflexus* (n=1), *Dermacentor reticulatus* (n=1), *Rhipicephalus bursa* (n=2), *Hyalomma lusitanicum* (n=4), *Hyalomma scupense* (n=2) and *Ixodes ricinus* (n=2). One of the *I. ricinus* lines, IRE/LULS55, bears some genetic similarity to ticks reported as *Ixodes inopinatus*. All the new cell lines are free of bacterial contamination.
Conclusion: These new cell lines greatly increase the options for *in vitro* isolation, propagation and

study of bacterial and viral pathogens and symbionts encountered in European tick species. All the cell lines, and training in their maintenance, are available from the TCB (contact tickcellbiobankenquiries@ liverpool.ac.uk).

P 012

Diversity of tick species carried by migratory birds coming from Africa and detection of tick-borne *Rickettsia* spp. and *Coxiella burnetii*

E. Mancuso[1,2], *L. Toma*[3], *M. Menegon*[3], *F. Casale*[3], *M. Di Luca*[3], *M. Di Domenico*[1], *L. Di Gialleonardo*[1] *C. Cammà*[1], *L. Serra*[4], *G. Di Donato*[1], *M. Goffredo*[1], *F. Monaco*[1]
[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy
[2]University of Urbino, Biomolecular Sciences, Urbino, Italy
[3]Istituto Superiore di Sanità, Rome, Italy
[4]Istituto Superiore per la Protezione e la Ricerca Ambientale, Area Avifauna Migratrice, Ozzano dell'Emilia (BO), Italy

The role of migratory birds in the spread of ticks and tick-borne pathogens (TBPs) along their migratory routes from Africa to Europe is increasingly emerging. Indeed, wild birds are recognised as hosts of several tick species which often carry infectious bacteria responsible for zoonotic diseases and climate change fosters their introduction and adaptation into the Mediterranean region. The aim of the study was to assess the possible introduction of exotic ticks carried by migratory birds approaching Italy from Africa and to detect the presence of *Rickettsia* species and *Coxiella burnettii* they may harbour. We show the outcome of a two-years study based on the sampling of ticks from migratory birds collected on Ventotene island, one of the most important migrants" stop-over sites in the Mediterranean. During 2021 and 2022 spring seasons approximately 33,100 birds, mainly Passeriformes, were captured and inspected for presence of parasites. Among them 551 were found parasitised by 1,430 ticks which were first identified by morphology and then by molecular tools when needed. DNA was individually extracted and tested by molecular methods for identification of *Rickettsia* spp., *R. aeschlimannii* and *C. burnettii*. The sample consisted of 10 different hard tick species, including some exotic ones such as *Amblyomma variegatum*, some others belonging to the *A. marmoreum* complex, *Hyalomma truncatum* and the most abundant *H. rufipes*. Additionally, two individuals of the soft tick *Argas africolumbae*, never detected in Italy before, were found on the same bird host. Molecular analysis revealed a prevalence higher than 50% for *R. aeschlimannii,* and sequencing results identified other rickettsiae, including *R. africae*. Interestingly, *C. burnettii* was found only in the two soft ticks. Our findings confirm the role of migratory birds in the spread of ticks and TBPs, suggesting the need for a continuous surveillance to monitor the potential introduction of emerging diseases into Europe.

P 013

A review of *canine babesiosis with an emphasis on sub-Saharan infection*

A. Leisewitz[1]
[1]Auburn University, Department of Clinical Sciences, Auburn, Alabama, United States

Dogs are commonly infected with a range of *Babesia* parasite species that cause a wide range of clinical disease. Many practitioners in the developed world may regard the disease as less common and caused by parasites that tend to result in less severe disease. In the developing world (sub-Saharan Africa in particular), the infection is very common in dogs and also the parasite is generally significantly more pathogenic than the more commonly written and spoken about species. Although *Babesia rossi* is a less appreciated cause of disease, because it occurs in a region of the world that is underdeveloped where dogs are often not presented for veterinary care, it is quite likely that this is a very significant and underrecognized cause of disease in dogs. The findings of the largest single cohort of 320 *B. rossi* mono-infected dogs was recently published and provided the clearest picture yet of the potentially fulminant nature of this infection. This allows a comparison of the disease phenotype caused by the most common babesia parasites infecting dogs, namely *B. canis, B. gibsoni* and *B. vogeli* which are more common off the African continent. Tick-vectored disease is growing in importance as climate change and environmental degradation change tick distribution ranges. Global dog movements pose a constant threat to the introduction of new diseases to places where these infections do not currently occur. Understanding parasite biology, disease pathogenesis and treatment and research priorities is crucial to staying abreast of this important infectious threat which is likely far more common in sub-Saharan Africa than in other developed parts of the world. This presentation will provide a description of the disease caused by *B. rossi* specifically and will also briefly compare the clinical diseases caused by *B. canis, B. gibsoni* and *B. vogeli* for comparison.

P 014

Genetic diversity of South African cattle hard-tick species (Acari: Ixodidae)

T. G. Makwarela[1], *N. O. Mapholi*[2], *T. Masebe*[2]
[1]Unisa, Life and consumer sciences, Florida, South Africa
[2]Unisa, Life and consumer sciences, Florida, South Africa

Introduction: Ticks of the Ixodidae family are essential pathogen vectors in human and animal health. Numerous tick species are grouped into species complexes due to their physical resemblance. South Africa is one country where cattle are targeted by a wide range of hard tick species. Using the 16s rDNA sequence genes, we were able to identify ticks infesting cattle in South Africa at the molecular level. **Methodology:** ±1893 hard ticks were collected from cattle in 23 locations throughout six provinces. Initially, morphological features were used to classify these ticks into species. After 16S rDNA amplification, all samples were positive with a size of 610 bp and had clear bands on a 2% agarose gel electrophoresis, and 250 PCR-positive samples were sent to the CAF DNA Sequencing Unit in

Stellenbosch (South Africa). A clustering analysis based on 16S rDNA sequences was performed using MEGA 11 to construct a Maximum Likelihood. Additional PCA was constructed using an R server for sanger sequence alignment.

Results: The use of the Maximum Likelihood method to create phylogenetic trees revealed that each species clustered into discrete clades, demonstrating the utility of rDNA genes for tick species identification. The phylogenetic study revealed the genera *Amblyomma*, *Hyalomma*, *Haemaphysalis*, *Ixodes*, and *Rhipicephalus*. When analysing intra- and interspecies K3P distances, most samples revealed that intraspecific distances ranged between 0 and 0.05.

Conclusion: The current work is the first to use sequencing analysis to identify tick species infesting cattle in South Africa and it is the first to report *Rh. glabroscutatus* in KwaZulu Natal and *A. hebraeum* in the Free State region. The presence of multiple phylogenetic groupings and significant mean and pairwise genetic distances suggests that there is biological diversity among hard ticks in the research area.

P 015

Avian migration as a biological highway – determining connectivity of tick-borne pathogen communities along avian migratory routes

R. E. Rollins[1], *M. Liedvogel*[1]
[1]Institute of Avian Research, Wilhelmshaven, Germany

Seasonal migration is a widespread phenomenon across all animal taxa, of which birds are one of the best studied groups. Bird migration can influence the transport and transmission of various pathogens as well as parasites, such as ticks, over large geographic distances and even between continents. Ticks are obligate, blood-sucking arthropods with multiple distinct life-stages and can be found on almost every continent. Many tick species utilize birds as hosts and in recent years many studies have highlighted the movement of non-endemic ticks on migratory birds. These ticks have further been shown to be positive for various exotic tick-borne pathogens. Only recently have there been reports of local transmission of various exotic tick-borne pathogens in Europe suggesting a potential for establishment, warranting further study. Helgoland is an island in the North Sea where one of the oldest ringing schemes in the world is located and where migratory behavior of birds has focally been monitored and studied for the last century. This work aims to harness the power of the long-term ringing study from the Helgoland study system to address three main goals: 1) Which birds are contributing the most to tick and tick-borne pathogen movement on Helgoland? 2) Where do these birds breed and overwinter? 3) Are the pathogen communities in these locations connected by the migratory routes and if so can exchange be quantified? Through integrating tick collection at overwintering sites, breeding sites, and from migratory birds we aim to quantify and better understand pathogen community exchange due to bird migration. Furthermore, the opportunity to present this project at ISTTBD-XV will allow for discussions on the proposed methods with experts regarding ticks and tick-borne pathogens.

P 016

High Lyme borreliosis (LB) disease burden in Germany demonstrated by the estimated incidence of symptomatic LB after adjusting for under-ascertainment by public health surveillance, 2021

J. Olsen[1], G. Brestrich[2], A. Pilz[3], K. Halsby[4], P. Kelly[1], C. Malerczyk[2], F. Angulo[1], J. Stark[1], J. Moisi[5] L. Jodar[1]
[1]Pfizer Vaccines, Medical and Scientific/Clinical Affairs, Collegeville, United States
[2]Pfizer Pharma GmbH, Pfizer Vaccines, Berlin, Germany
[3]Pfizer Corporation Austria, Pfizer Vaccines, Vienna, Austria
[4]Pfizer Vaccines, Medical and Scientific/Clinical Affairs, London, United Kingdom
[5]Medical and Scientific/Clinical Affairs, Pfizer Vaccines, Paris, France

Background: Lyme borreliosis (LB) is endemic in many European countries. Although many European countries conduct public health surveillance for LB, symptomatic LB may be under-ascertained by surveillance. Nine of sixteen German states (with 42% of the national population) conduct public health LB surveillance with mandatory notification by clinicians and laboratories. The extent of under-ascertainment of symptomatic LB cases by surveillance in Germany has not been reported.
Methods: In German states that conduct LB surveillance, the incidence of symptomatic LB was estimated based on literature-reported *Borrelia burgdorferi* sensu lato seroprevalence, a 50% asymptomatic proportion, and a 10-year duration of antibody detection. The estimated number of symptomatic LB cases was then compared to the number of surveillance-reported cases to derive under-ascertainment multipliers in children and adults. These multipliers were applied to the number of surveillance-reported cases to estimate the number and incidence of symptomatic LB cases in 2021. A sensitivity analysis evaluated the impact of 20-year and 5-year duration of antibody detection on multipliers and incidence.
Results: The estimated number (incidence) of symptomatic LB cases in states that conducted surveillance was 128,870 (408/100,000) in 2021 or 12 symptomatic LB cases for each of the 11,051 (34/100,000) surveillance-reported cases. In the sensitivity analysis, the estimated number (incidence) of symptomatic LB cases in states that conducted surveillance was 64,935–259,740 (204-816/100,000).
Discussion: Although Germany has a high incidence of LB, there is substantial under-ascertainment of symptomatic LD cases by public health surveillance. Public health authorities should consider the impact of under-ascertainment when considering the disease burden of LB. Enhancements of LB surveillance, including nationwide expansion, would facilitate further elucidation of the true LB disease burden in Germany.

P 017

Genomic characterisation of four new Varanid-associated *Borrelia* spp. from Australia and Indonesia

A. Gofton[1], *S. Supriyono*[2], *A. Takano*[3]
[1]CSIRO, Health and Biosecurity, Canberra, Australia
[2]IPB University, Faculti of Veterinary Medicine, Bogor, Indonesia
[3]Yamaguchi University, Faculty of Veterinary Epidemiology, Yamaguchi, Japan

Question: The reptile-associated (RA) *Borrelia* clade represents a distinct evolutionary lineage and includes novel species found in diverse hosts, including tortoise, snakes, and Varanid lizards. However, most RA *Borrelia* remain poorly characterised, and we lack a thorough understanding of their ecology, biology, genomic architecture, and evolutionary history.

Methods: We sequenced the first Varanid-associate (VA) *Borrelia* genomes from four novel isolates from *Amblyomma* and *Bothriocroton* ticks from Indonesia and Australia. Oxford Nanopore and Illumina sequencing produced high-quality genomes including plasmids, which were used in comparative genomic analyses to investigate their genomic structure and evolutionary relationships.

Results: The genomes of VA borrelia retain the conserved and highly syntenic chromosome found in other *Borrelia* sp., and a variable number of linear and circular plasmids which are highly variable even between closely related strains. VA genomes all included a megaplasmid which is conserved among all RA and relapsing fever (RF) borreliae, however, their remaining plasmids have a high degree of dissimilarity from other borreliae and many represent unique RA-specific plasmid types and may encode genes important for reptile-specific host adaptations. Phylogenomic analyses based on whole genome orthologs and marker genes reinforces the distinctness of RA *Borrelia* from the RF and Lyme-borreliosis clades, and demonstrates a pattern of host-driven RA *Borrelia* co-evolution, with distinct monophyletic sub-lineages associated with different host types.

Conclusions: RA *Borrelia* spp. genomes contain unique genomic features that are likely driven by host adaptation. Although this group of borreliae does not appear to have human-disease associations, they are an important group from which we may glean significant insights into the evolutionary history of *Borrelia* including the influence of host and vector adaptation on early *Borrelia* diversification.

P 018

Molecular detection of *Anaplasma phagocytophilum* in dogs and horses from Germany (2008–2021)

I. Schäfer[1], S. Fischer[2], C. Silaghi[2], B. Kohn[3], C. Marsboom[4], G. Hendrickx[4], H. Gehlen[5], E. Müller[1]
[1]Laboklin GmbH & Co. KG, Department of Clinical Pathology, Bad Kissingen, Germany
[2]Friedrich-Loeffler-Institute, Institute of Infectology, Greifswald-Insel Riems, Germany
[3]Freie Universität Berlin, Faculty of Veterinary Medicine, Clinic for Small Animals, Berlin, Germany
[4]Avia-GIS R&D department, Zoersel, Belgium
[5]Freie Universität Berlin, Faculty of Veterinary Medicine, Clinic for Horses, Berlin, Germany

Introduction: *Anaplasma* (*A.*) *phagocytophilum* is an obligate intracellular bacterium causing granulocytic anaplasmosis. Ticks of the *Ixodes persulcatus* complex are the primary vectors.
Objectives: Aim of the study was to assess the percentage of positive test results for *A. phagocytophilum* in dogs and horses and to identify potential risk factors for infection.
Materials & methods: Results of PCR-testing were included in this retrospective study. Tests requested by German veterinarians for dogs and horses between 2008 and 2021 were performed in the laboratory LABOKLIN.
Results: In total, 1,517/32,250 dogs (4.7%) and 190/1,246 horses (15.2%) tested positive. Seasonality had a statistically significant impact in both species ($P < 0.001$ each) with highest percentage of positive tested dogs (8.9%, $\mathrm{OR} = 3.46$) and horses (25.2%, $\mathrm{OR} = 3.28$) in summer and especially in June (dogs: 11.7%, horses: 33.2%). Regionality had a statistically significant impact on PCR-results comparing northern federal states to the others in dogs ($P < 0.001$, $\mathrm{OR} = 1.27$) and horses ($P < 0.001$, $\mathrm{OR} = 2.69$), as well as male sex in dogs ($P < 0.001$, $\mathrm{OR} = 1.24$). Years of testing (dogs: $P = 0.071$, horses: $P = 0.067$) and male sex in horses ($P = 0.283$) had no statistical significance.
Conclusion: Seasonality in accordance with peaks in vector activity as well as regionality contributed to positive PCR-results in dogs and horses. Male sex increased the likelihood in dogs. There was a trend of rising importance of *A. phagocytophilum* infections. Horses showed higher percentage of positive PCR-results compared to dogs, maybe due to lack of ectoparasite prophylaxis.

P 019

Detection of tick-borne diseases in horses from Lithuania by serological tests and blood smear microscopy

D. Mikalauskienė[1], Z. Mikniene[1], D. Bajorinė[1]
[1]Lithuanian University of Health Sciences, Large animal clinic, Kaunas, Lithuania

Ticks are vectors of several infectious, equine diseases, including equine granulocytic anaplasmosis (EGA) and equine piroplasmosis (EP), that were the main subject in our research. Many studies predict expansion of tick habitats to the cooler climate zones. Our research took place in Lithuania, which is located in a temperate climate zone where equine tick-borne diseases are still diagnosed rarely.

We studied 36 horses during the spring season when the tick population increases the most. Out of 36 specimens, EGA was diagnosed in six horses (16.67%) using both peripheral blood smear (PBS) evaluation and lateral flow immunoassays (LFIAs) rapid tests, while EP was diagnosed in three horses (8.33%) using only PBS evaluation. All the horses diagnosed with EP had no clinical symptoms and these findings were accidental. 33.33% of specimens tested positive for EP had clinical symptoms, all of them showed positive results in both evaluation of PBS and LFIAs. Surprisingly, 18.18% of animals with no clinical symptoms tested positive for these tick-borne diseases. Four out of six asymptomatic EGA samples tested positive with both LFIAs and PBS microscopy methods. There was one sample that only tested positive with LFIAs and one that tested positive only using microscopic evaluation of PBS.

The current findings can be used in epidemiological studies of tick-borne equine diseases in the geographic area. We hypothesize that there are more undiagnosed EP and EGA cases than diagnosed. Further research is needed to determine the current situation of equine tick-borne diseases in temperate climate zones and to analyse climate change impact on the topic. Our results suggest that neither LFIAs or PBS microscopy are completely reliable on their own as the pathogen can be detected with different methods depending on its life cycle and pathogenesis during testing, therefore we advocate the use of both methods simultaneously in clinical practice.

P 020

Lyme disease seroprevalence and larvae infestation with *B. burgdorferi* in Bulgarian songbirds

I. Trifonova[1], *I. Christova*[1], *N. Ivanova-Aleksandrova*[1], *T. Gladnishka*[1], *V. Ivanova*[1], *K. Ngoc*[1]
E. Taseva[1], *E. Panayotova*[1]
[1]National Centre Of Infectious And Parasitic Diseases, Microbiology, Sofia, Bulgaria

Introduction: Birds are known to be hosts for the immature stages (nymphs and larvae) of the vector *I. ricinus*. The development of high bacteremia in blood and the lack of serious clinical course in birds define them as a competent reservoir for the causative agents of infection –*B. burgdorferi* s.l. Both species *Turdus merula* and *Parus major* are among most abundant avian reservoirs for *B. burgdorferi* in Europe.
Objectives: To study the presence of infection with the causative agent of Lyme disease and seroprevalence among two species of songbirds in the region of Sofia
Materials & methods: The avian were caught with ornithological nets; ticks on them were removed and *I. ricinus* larvae (n=48) were proceeded for investigation; serum samples (n=108) were taken from species *T. merula* (n=69) and *P. major (n=39)*. ELISA test for specific IgG antibodies and nested PCR to detect larvae infestation with *B. burgdorferi* s.l. were applied.
Results: For the study period (June 2021–June 2022), 16% (8/48) of the *I. ricinus* larvae were confirmed to be positive for *B. burgdorferi* s.l. by nested PCR. All infested larvae were removed from *T. merula*. ELISA test detected specific IgG antibodies against *B. burgdorferi* s.l. in 37% (40/108) of the birds. Despite the higher seroprevalense in *P. major* – 41% (28/69) in comparison with *T. merula* – 31% (12/39), the difference was not statistically significant ($p>0.05$).

Conclusion: The presence of specific antibodies confirms active circulation of *B. burgdorferi* s.l. in both species *T. merula* and *P. major*. Since the transovarial transmission of *B. burgdorferi* s.l. in Ixodid ticks is very rare, we considered that the infestation of the larvae is from the bird host. We found out relatively high levels of infestation with *B. burgdorferi* in larvae from *T. merula*, which confirms the contribution and the importance of species in the transmission cycle of Lyme borreliosis in Bulgaria. This study was supported by project BG05M2OP001-1.002-0001.

P 021

Evaluation of two commercial ELISAs for the detection of antibodies against the tick-borne pathogen *Borrelia miyamotoi*.

O. Stukolova[1], *M. Sokolova*[1], *D. Sarksyan*[2], *N. Kolyasnikova*[1,3], *L. Karan*[1]
[1]CRIE, Moscow, Russian Federation
[2]Izhevsk State Medical Academy, Izhevsk, Russian Federation
[3]Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Sciences, Moscow, Russian Federation

Introduction: Previously it was shown that antibodies in serum of patients with *Borrelia miyamotoi*-caused disease (BDM) may cross-react in commercially available Lyme borreliosis diagnostic tests due to high homology in a number of antigenic proteins of *B.miyamotoi* and *B.burgdorferi s.l.* Objectives: Here we aimed to evaluate the possibility to discove IgM or IgG in BMD-patients serum samples with help of two commercial ELISAs kits both of which are often used for Lyme disease diagnostics in Russia and based on mixture of a number of recombinant proteins of *Borrelia burdorferi s.l.* spices.
Material & Methods: 254 serum samples were obtained during prospective study (day 0 – 499 from symptom onset) from 82 patients with PCR-confirmed BMD (Amplisens *Borrelia miyamotoi* FL, CRIE, Russia). 75 health blood donors serum samples were collected on non-endemic territory of Volgograd region of Russia. ELISA kits Lyme Best IgG/IgM (Vector Best, Russia) and recomwell Borrelia IgG/IgM (Mikrogen Gmbh, Germany) were used off-label due to their antigenic composition.
Results: LymeBest IgM and recomwellBorreia IgM had sensitivity of 46% and 20% respectively in serum samples of BDM patients collected on day 7-28 after symptom onset and specificity of 88% and 100% in control group. LymeBest IgG and recomwellBorrelia IgG had sensitivity of 12% and 4% respectively in serum samples of BDM patients collected on day 20–378 after symptom onset and specificity of 93% and 97% in control group. Combined IgM/IgG sensitivity and specificity were 50% and 88% respectively for LymeBest kits and 20% and 98% respectively for recomwellBorrelia kits.
Conclusion: Unlike C6 ELISA (Immutetics, USA) previously proved to have sensitivity of 76% when BMD patients samples were studied [1], kits used in this study shown low sensitivity in case of BMD. Hence BMD could be miss-diagnosed when ELISA kits based on *B.burgdorferi s.l.* proteins are used.
1 Koetsveld et al Microbiol Infect 2019

P 022

Borrelia burgdorferi sensu lato and other tick-borne pathogens in *Ixodes ricinus* ticks in urban green area in Prague

E. Richtrová[1], *K. Kybicová*[1], *P. Míchalová*[1], *A. Lukavská*[1], *J. Navrátil*[1]
[1]National Institute of Public Health, National Reference Laboratory for Lyme Borreliosis, Prague, Czech Republic

Ixodes ricinus ticks are considered the vectors of the *Borrelia burgdorferi* sensu lato complex and other tick-borne pathogens such as *Anaplasma phagocytophilum*, *Rickettsia* spp. and *Babesia* spp. in urban areas, including city parks and green recreational areas. The aim of the present study was to determine the prevalence of these pathogens in urban areas in the city of Prague, Czech Republic. In total, 3856 ticks *I. ricinus* collected in selected public green areas in Prague during spring 2014 to 2022 were individually analyzed by a quantitative real time PCR. Ticks were infected with at least one pathogen in 36.7%. 28.2% of *I. ricinus* ticks were tested positive for *B. burgdorferi* s. l., 5.4% for *A. phagocytophilum*, 3.3% for *Rickettsia* spp. and 2.5% for *Babesia* spp. Co-infections occurred in ticks in all years on average in 3%. The prevalence of *Borrelia* infection varied significantly ($p<0.01$) between collection sites and the highest numbers of infected ticks were found in the central city areas as well as *Rickettsia* spp. positive ticks. On the other way, the number of positive ticks for *A. phagocytophilum* and *Babesia* spp. was higher in the city parks situated at the edge of the city, which are not surrounded by buildings. Places serving people for recreational and sport activities in urban areas are characterized by a lower diversity of reservoir hosts, provide opportunity for exposure to *Borrelia*-infected ticks, and pose a higher infection risk.

Our results add to the current knowledge about *Borrelia* and other tick-borne pathogens in urban areas in the Czech Republic and reveal the potential health risk for people and animals. This study highlights the need for the medical and veterinary attention and prevention of people and animals visiting these areas.

This research was supported by MH CZ-DRO (The National Institute of Public Health – NIPH, 75010330).

P 023

Evaluation of undertesting and underdiagnosis of tick-borne encephalitis in Germany

K. Schley[1], *J. Friedrich*[1], *A. Pilz*[2], *L. Huang*[3], *B. Balkaran*[4], *M. C. Maculaitis*[4], *C. Malerczyk*[1]
[1]Pfizer Pharma GmbH, Berlin, Germany
[2]Pfizer Corporation, Wein, Austria
[3]Pfizer, Inc, New York, United States
[4]Cerner Enviza, Malvern, United States

Question: Tick-borne encephalitis (TBE) is a viral infectious disease involving the central nervous system (CNS) that can lead to long-term neurological symptoms. Undertesting and underdiagnosis occur because TBE can present with nonspecific symptoms, but even TBE with typical symptoms may be missed due to insufficient disease awareness and lack of routine screening. This study evaluated real-world TBE laboratory testing rates across Germany.

Methods: This retrospective, mixed methodological study included qualitative interviews with 12 physicians on TBE decision-making, laboratory testing and diagnostic behavior; 166 physicians participated in a quantitative review of medical charts (N=1400). Hospital-based physicians managing and ordering laboratory tests within the past year were included if they specialized in infectious disease, intensive care unit, emergency room, neurology, or pediatrics. Data were analyzed using descriptive statistics and patient charts were used to evaluate TBE testing and positive rates. Analyses were stratified by symptoms and tick bite status.

Results: TBE cases were highest in patients with meningitis-only symptoms. Risk factors most frequently reported were outdoor activities in forests or grassy areas (39.9%), traveling (22.9%), and tick bite (22.1%). TBE testing rates ranged from 54.0% (nonspecific neurological symptoms [NNS]) to 65.6% (encephalitis only), and TBE positive rates ranged from 5.3% (NNS) to 36.9% (meningitis only). Patients with headache, high fever, flu-like, or previous tick bite and CNS symptoms had higher TBE testing rates than those without.

Conclusions: TBE undertesting is common in Germany, even among patients with typical symptoms, thus potentially leading to underdiagnosis, underreporting, and inaccurate representation of areas at risk of TBE. Thus, laboratory testing should be included in routine practice when patients display typical TBE symptoms or are exposed to common risk factors. This study was funded by Pfizer Inc.

P 024

Iflaviruses in *Ixodes* spp. cell lines – a cautionary tale

A. M. Palomar[1,2], J. J. Khoo[1], L. Bell-Sakyi[1]
[1]Institute of Infection, Veterinary and Ecological Sciences/University of Liverpool, Liverpool, United Kingdom
[2]Centre of Rickettsiosis and Arthropod-Borne Diseases, Hospital Universitario San Pedro-CIBIR, Logroño, La Rioja, Spain

Introduction: Ticks harbour viruses belonging to a range of families, encompassing both arboviruses transmitted between ticks and their vertebrate hosts and tick-only viruses. Among the latter, several iflaviruses (family *Iflaviridae*, order *Picornavirales*) have recently been found to infect hard ticks including *Ixodes ricinus*. Surprisingly, an iflavirus (ISIV) reported from the *Ixodes scapularis* cell line ISE6 maintained in Japan was found to be nearly identical to the *I. ricinus* iflavirus. The Tick Cell Biobank (TCB) houses ISE6, four other *I. scapularis* cell lines and five cell lines derived from European *I. ricinus*, one of which (IRE11) was generated in the same laboratory as the *I. scapularis* lines.
Objective: To investigate the origin of ISIV, we screened the *Ixodes* spp. cell lines for its presence.
Materials and Methods: RNA extracted from growing cells in 2011 and 2022 was retrotranscribed and PCR-screened using primers targeting ISIV polyprotein. PCR products of the expected size (437 bp) were sequenced.
Results: A sequence amplified from ISE6 and IRE11 cells was >99% identicalto the published ISIV sequence, and >97% identical to a published sequence from *I. ricinus*. A sequence >99% identical to ISIV was also amplified from the *I scapularis* cell line IDE8 received by the TCB in 2001, but not from lower passage cells received in 1997 and resuscitated in 2022. None of the other cell lines yielded PCR products.
Conclusions: Presence in the *I. scapularis* lines ISE6 and IDE8 of the iflavirus sequence associated with IRE11 cells probably resulted from cross-contamination events. In the case of IDE8, this probably occurred after initiation of the IRE11 cell line, as IDE8 cells prior to the initiation of IRE11 did not harbour the iflavirus. The significance of this example of likely cross-contamination for users of tick and, more widely, arthropod cell lines that support persistent infection with viruses in the absence of any apparent cytopathic effect, is discussed.

P 025

Borrelia spp. and *Anaplasma phagocytophilum* infections in ticks from urban parks of Lithuania

J. Snegiriovaitė[1], J. Radzijevskaja[1], A. Paulauskas[1]
[1]Vytautas Magnus university, Biology, Kaunas, Lithuania

In many European cities, the abundance of ixodid ticks and the cases of diagnosed tick-borne diseases have been expanding. The transformation of natural ecosystems into urban areas becomes a major problem because it affects the circulation of tick-borne pathogens in cities and increases the human population's exposure to ticks. Lyme borreliosis (LB) is the most common tick-borne infectious disease in Lithuania, and the morbidity of LB during the past decade has particularly increased in cities. However, to date, comprehensive data on the abundance of ticks and the prevalence of causative agents of LB and other tick-borne pathogens (TBPs) in urban areas of Lithuania remain largely unknown and still need to be discovered. This study aimed to investigate the prevalence of *Borrelia* spp. and *Anaplasma phagocytophilum* in ticks from Lithuanian urban areas. A total of 526 questing *Ixodes ricinus* ticks were collected using a flagging method in fifteen parks in eight Lithuanian cities during spring and autumn in 2021 and 2022. Ticks were screened for the presence of pathogens using duplex real-time PCR assay amplifying *Borrelia* 16S rRNA and *A. phagocytophilum msp4* genes. Multilocus sequence analysis using nested PCRs and PCR product sequencing were performed to identify pathogen species and strains. The overall infection rate with *Borrelia* spirochaetes in ticks was 35% (184/526) and varied from 0% to 58.1 % in different parks. *A. phagocytophilum* DNA was detected in 5.7% (30/526) ticks. Coinfection with *Borrelia* spp. and *A. phagocytophilum* was detected in ten ticks. Sequencing revealed the presence of four *Borrelia* species: *B. afzelii*, *B. garinii*, *B. burgdorferi* s.s. and *B. miyamotoi*. This study reports new data on TBPs in urban zones in Lithuania and demonstrates a high risk of encountering tick-borne pathogens in cities.

P 026

Babesia canis strains circulating in dogs in Lithuania

J. Radzijevskaja[1]*, A. Paulauskas*[1]*, D. Mardosaitė-Busaitienė*[1]*, A. Aleksandravičienė*[1]*, B. Karvelienė*[2]
[1]Vytautas Magnus University, Biology, Kaunas, Lithuania
[2]Lithuanian University of Health Sciences, Faculty of Veterinary Medicine, Kaunas, Lithuania

Question. An emerging and rapidly expanding tick-borne infectious disease, canine babesiosis caused by *Babesia canis* is a major veterinary problem in central and northeastern Europe, including Lithuania. The emergence of *B. canis* is mainly associated with the expanding range of its primary vector, the *Dermacentor reticulatus* tick, environmental changes, and migrations of humans and animals. Genetic variability is important for the survival of *Babesia* parasites in their vertebrate hosts and is associated with the difference in the virulence of *B. canis* strains. The distribution and prevalence of *B. canis* genotypes differ between European regions. The aim of this study was to investigate the genetic diversity of *B. canis* strains isolated from naturally infected dogs in Lithuania.
Methods. Blood samples were collected from 149 dogs with a presumptive diagnosis of babesiosis in six regions of Lithuania. PCR-RFLP and sequence analysis of 18S rRNA and *Bc28*.1 genes were performed.
Results. Based on 18S rRNA PCR-RFLP profiles and sequence analysis, three *B. canis* genotypes and five sequence variants were identified. Based on the PCR-RFLP profiles of the *Bc28.1* gene, four *B. canis* genotypes were identified. Sequence analysis of the *Bc28.1* gene revealed 18 polymorphic sites and 13 sequence variants among the Lithuanian samples. The molecular characterisation of *B. canis* 18S rRNA and *Bc28.1* genes indicated the presence of genetically heterogenic *B. canis* strains in Lithuania. The *B. canis* genotypes obtained were detected with different prevalence rates in different regions of Lithuania.

Conclusion. The results of the present study show that the distribution and prevalence of *B. canis* Bc28.1 genotypes in Lithuania are specific and differ from those obtained in northeastern and central Europe. Local ecological factors may affect the distribution and survival of particular *B. canis* genotypes associated with genetically different *D. reticulatus* populations.

P 027

First results of a biological control measure of a TBEV focus in Germany

A. Lindau[1]*, K. Fachet*[1]*, F. Greiner*[1]*, M. Wassermann*[1]*, U. Mackenstedt*[1]
[1]University of Hohenheim, Department of Parasitology, Stuttgart, Germany

Introduction: TBE is the most important tick-borne viral infection in Europe. TBEV-infected ticks and rodents occur in nature only in very confined areas. These TBEV foci are often less than 100 m2 in size. This restriction to a relatively small area opens the possibility of treatment/control.
Objective: The present study aims to investigate the suitability of the entomopathogenic fungus *Metarhizium pemphigi* and its application with rodent nesting material for biological control of ticks and thus the TBE focus.
Materials andmethods: A known TBE focus in a private garden was used as study site. Prior to treatment, the local rodent population was serologically tested for TBEV antibodies. The target of the treatment were the ticks present in the rodent burrows. For this reason, cotton was treated with conidia of *M. pemphige* and placed in cardboard rolls. Five rolls were distributed on the ground in the garden and the cotton was offered to the rodents as nesting material. The rolls were replaced once per month from August to November 2021.
In 2022, small rodents were again trapped in the garden, sampled, and tested for TBEV antibodies. Ticks were also collected from the garden and surrounding area and tested for TBE virus using RT-qrt-PCR.
Results: A total of 22 rodents were sampled in 2021, identified as *Myodes glareolus* (5 individuals), *Apodemus flavicollis* (13 individuals), and *Apodemus sylvaticus* (4 individuals). TBEV antibodies were detected in 5 *A. flavicollis* mice (22.7%).
In 2022, 23 different rodents were captured and sampled (14 *M. glareolus*, 8 *A. flavicollis*, 1 *A. sylvaticus*). No TBEV antibodies were detectable in these animals. In addition, 151 *Ixodes ricinus* were collected inside the garden and 2826 *I. ricinus* in surrounding areas. TBEV was not detected in any of these ticks.
Conclusion: It appears that the release of *M. pemphigi* via rodent nesting material is an appropriate method to eliminate TBEV in a focal area, at least temporarily.

P 028
Hybrid capture-based next generation sequencing of *Borrelia* in the United Kingdom

D. Carter[1], *D. Bailey*[2], *J. Duggan*[3], *N. Evans*[4], *S. Pullan*[1]
[1]UK Health Security Agency, Pathogen Genomics, Porton Down, United Kingdom
[2]UK Health Security Agency, Diagnostic Support, Porton Down, United Kingdom
[3]UK Health Security Agency, Rare and Imported Pathogens, Porton Down, United Kingdom
[4]University of Liverpool, Dept of Infection Biology and Microbiomes, Liverpool, United Kingdom

A key challenge of generating whole genome sequences of organisms such as *Borrelia* directly from patient samples or tick extracts is the low abundance of target DNA and the high abundance of host (e.g. human or tick) material.

Propagation of bacteria using solid or liquid culture media is often used to amplify the target organism in order to generate enough starting material to successfully generate whole genome sequences, however key plasmids are often lost during this process due to the lack of selective pressures the organism would encounter during survival in a vector or infection in a host.

PCR amplification is also commonly used to amplify key regions of the target genome to allow genotyping of the organism and to provide enhanced data such as the presence or absence of antibiotic resistance genes, however this method only provides information about these target regions and this method is susceptible to issues such as mutations in primer binding sites.

As an alternative to culture and PCR, hybrid capture-based target enrichment allows the enrichment and amplification of target genomes from mixed samples such as patient biopsies and tick extracts and has previously been used to generate whole genome sequences of *B. burgdorferi s.s*. in the United States (Carpi et al., 2015). For this project, a panel of biotynilated probes were designed specifically focussing on *B. garinii* and *B. afzelii* which are more prevalent in the UK and a range of cultured isolates and tick extracts were sequenced using this method.

Generating additional whole genome sequences of UK *Borrelia* will allow a detailed comparison of different *Borrelia* genotypes found in different regions of the UK and oversees and allow us to determine the suitability of commercially available diagnostic assays including those using recombinant antigens to detect and characterise infections caused by UK *Borrelia*.

P 029

Erythema migrans in splenectomised patients

V. Maraspin[1], P. Bogovič[1], T. Rojko[1], K. Ogrinc[1], E. Ružić-Sabljić[2], F. Strle[1]
[1]University Medical Center Ljubljana, Department of Infectious Diseases, Ljubljana, Slovenia
[2]Institute of Microbiology and Immunology, Medical Faculty, University od Ljubljana, Ljubljana, Slovenia

Introduction: Information on the course and outcome of erythema migrans (EM) in splenectomised patients is lacking.

Methods: We compared 33 splenectomised patients (8 females and 25 males, aged 51 (17–70) years), diagnosed with EM at a single centre during 1994–2020, with 66 age-, sex-, and antibiotic therapy-matched patients without splenectomy, diagnosed with EM in the same year. All patients were prescribed antibiotics routinely used for EM and were followed for one year.

Results: At admission, 7/33 (21%) splenectomised patients had indications of early disseminated LB: 3 had multiple EM, 3 pronounced newly developed symptoms accompanying EM, one 2nd degree atrioventricular block. Comparison of pre-treatment characteristics revealed that splenectomised patients had shorter duration of EM before diagnosis (5 vs 7.5 days; $p=0.017$) and smaller diameter of EM (11 vs 14 cm; $p=0.056$), more often reported constitutional symptoms (45% vs 21%, $p=0.024$) including headache and fever, more often had elevated (>10x109/L) blood leukocyte count (6/33, 18% vs 2/66, 3%; $p=0.018$) and more frequently showed symptoms/signs of disseminated LB (21% vs 0%, $p=0.001$). Treatment failure was registered in 5/33 (15%) splenectomised patients and in 0/66 patients representing control group ($p=0.003$). In two patients EM remained visible >2 months after antibiotic treatment, in one borreliae were demonstrated in culture of skin obtained at the site of previous EM 2 months after antibiotic therapy, while two patients developed pronounced constitutional symptoms 5 months after initial visit. All the patients received an alternative antibiotic, had complete resolution of symptoms/signs and favourable outcome of the illness.

Conclusions: Splenectomised patients with EM have more often symptoms/signs of disseminated Lyme borreliosis at presentation as well as treatment failure than non-splenectomised patients with EM. However, retreatment with antibiotics results in favourable outcome of the illness.

P 030

Influence of trails on vertebrate and tick communities in a preserved area of Atlantic Forest in southern Brazil

A. Suzin[1], M. H. Tognolli[2], A. Vogliotti[3], S. F. Adami[3], W. A. de Castro[3], V. Ramos[1], G. V. Tolesano Pascoli[1], K. Torga dos Santos[1], M. X. da Silva[4], P. H. Nunes[3], M. P. J. Szabó[5,1]
[1]Universidade Federal de Uberlândia, Laboratório de Ixodologia, Uberlândia, Brazil
[2]Universidade Estadual de Campinas, Campinas, Brazil
[3]Universidade Federal da Integração Latino-Americana, Foz do Iguaçu, Brazil
[4]Projeto Carnívoros do Iguaçu no Parque Nacional do Iguaçu, Foz do Iguaçu, Brazil
[5]Universidade Federal de Uberlândia, Veterinary Pathology, Uberlândia, Brazil

Introduction: The ecological patterns that shape the spatial distribution of ticks according to host availability in untouched and altered environments in tropical forests are poorly understood. We herein compared the vertebrate and tick communities of open trails (OT) and control transects (CT) in a preserved Atlantic Forest, the Iguaçu National Park (INP), between March 2019 and January 2020.
Objectives: Our aim was to verify whether tick distribution over time is determined by the type of environment, by vertebrate activity, and to compare the structure and composition of vertebrate and tick communities between OTs and CTs.
Methods: Vertebrates were monitored using camera traps and ticks collected using flannel dragging.
Results: The number of records of vertebrates (n = 5,058) was similar in the OTs and CTs (49.6% and 50.4%, respectively). However, at the order level, carnivores and lagomorphs were more recorded in OTs and rodents in CTs. The number of ticks and stages did not differ between OTs and CTs, however, nymphs of *Amblyomma coelebs* were more abundant in OTs and nymphs and adults of *Amblyomma brasiliense* in CTs. The Shannon diversity of mammals was higher in the OTs and the beta diversity for all evaluated groups was similar between the two types of environments with high similarity in composition. Tick abundance did not increase with host activity.
Conclusions: Our results indicate that some taxa of vertebrates and ticks use the Atlantic Forest environment differently and future work should investigate in detail habitat selection in the environment-tick-host relationship. Knowledge of these aspects in pristine areas helps to understand the ecological history of ticks and deviations that lead to population overgrowth of vectors and tick-borne diseases in anthropized areas.

Financial support: CAPES, CNPq, FAPEMIG

P 031

Automating of an *ex vivo* feeding system for tick research

D. Hartmann[1]*, T. Hatalová*[1]*, M. Kucera*[1]*, P. Kopáček*[1]*, J. Perner*[1]
[1]Biology Centre of the Czech Academy of Sciences, Parasitology, Ceske Budejovice, Czech Republic

Introduction: Ticks, notorious disease vectors, are obligatory blood feeders and interfere with host immune and haemostatic systems, often for days, by secreting bioactive molecules in their saliva as well as serving as vehicles for pathogen transmission. The inevitable association of ticks and laboratory animals, however, has never allowed a dynamic analysis of tick secretory biomolecules as well as pathogens.
Objectives: Our proposed objective is the development of a novel fluidic design of ex vivo membrane feeding platform for ticks. Fluidic approaches offer a systems-level understanding of biological problems. While the approach has been successfully applied across various scales (nano-, micro-) to produce cells- and tissues-on-a-chip, very rarely has it been applied to organism-scale research. This proposal will practically devise a tick-on-a-chip platform to power novel insight into tick biology, inherently linked to their blood-feeding and pathogen transmission capacity.
Materials & methods: Feeders 3D-printed from UV-curable resin are connected to a blood flow propelled by a syringe pump or a combination of multiple pumps. Adult *Ixodes ricinus* ticks are used as pilot species.
Results: Compared to the static platform of the membrane feeding of ticks according to the protocol of Thomas Kroeber and Patrick Guerin, which requires a 12-h interval for the blood exchange, this novel solution is automated and requires significantly less personnel labour. The feeding performance of ticks on this novel fluidic system is currently being evaluated. Modulation of blood meal composition has been carried out to widen the applicability of the system (e.g. collection of tick saliva into protein-free meal).
Conclusion: This technological advancement opens up a new experimental platform that might be applicable in many tick research laboratories.

P 032

Seroprevalence of tick-borne encephalitis (TBE) virus antibodies in wild rodents from two natural TBE foci in Bavaria, Germany

P. J. Brandenburg[1]*, A. Obiegala*[1]*, H. M. Schmuck*[1]*, G. Dobler*[2]*, L. Chitimia-Dobler*[2]*, M. Pfeffer*[1]
[1]University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany
[2]Bundeswehr Institute of Microbiology, Departement of Virology and Rickettsiology, Munich, Germany

Tick-borne encephalitis (TBE) is the most important tick-borne viral disease in Eurasia. Rodents play an important role as natural hosts. Longitudinal studies on the dynamics of the seroprevalence rates in wild rodents in natural foci over the year are rare. Therefore, rodents were captured in two well-known natural foci in Bavaria, Germany, from March to October 2019 – 2022 in a capture-mark-release-recapture-study to collect blood or thoracic lavage samples. Overall, 651 samples from 478 small mammals (*Clethrionomys glareolus* and *Apodemus flavicollis*) were examined for antibodies against TBE virus by indirect immunofluorescence assay (IIFA). Furthermore, a generalized linear mixed model (GLMM) analysis was performed to investigate ecological as well as individual factors for the probability of infection in rodents. *Cl. glareolus* (19.4%) had a higher seroprevalence than *A. flavicollis* (10.5%). Within *Cl. glareolus* more males (40.4%) than females (15.6%) were affected and more adults (25.4%) than juveniles (9.8%). The probability of infection of rodents rather depends on factors such as species, sex, and age than on study site of a natural focus, year, and season. The high incidence rates of rodents, particularly male adult *Cl. glareolus*, highlight their important role in the transmission cycle of TBE virus in a natural focus and demonstrate that serologically positive rodents can be reliably detected in a natural focus regardless of the season or year.

P 033

Serological survey on canine and equine anaplasmosis and borreliosis in animals in contact with vulnerable people in Italy

D. Traversa[1], *P. Milillo*[2], *R. Maggi*[3], *G. Simonato*[4], *A. Di Cesare*[1], *C. Pezzuto*[5], *M. Grillini*[4], *S. Morelli*[1] *M. Colombo*[1], *A. Passarelli*[6], *A. Grassano*[7], *P. Serio*[2], *M. Losurdo*[2], *R. Brueckmann*[8]
[1]University of Teramo, Department of Veterinary Medicine, Teramo, Italy
[2]Azienda Sanitaria Locale (A.S.L.), Matera, Italy
[3]Freelance Veterinary Practicioner, Rome, Italy
[4]University of Padua, Animal Health, Padua, Italy
[5]Ambulatorio Veterinario Pezzuto Carlo/Piano Noemi, Campobasso, Italy
[6]Clinica Veterinaria Città di Bari, Bari, Italy
[7]Freelance Veterinary Practicioner, Matera, Italy
[8]EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany

Introduction: The zoonotic tick-borne pathogens (TBPs) *Anaplasma phagocytophilum* and *Borrelia burgdorferi s.l.* cause important diseases in dogs, horses, and people. Therefore, surveillance on the seroexposure to these TBPs is crucial under epidemiological and public health perspectives.
Objectives: The present Study has evaluated the seropositivity for *Anaplasma* and *Borrelia* of dogs and horses in contact with people at risk of developing severe anaplasmosis and/or borreliosis, with the aim to investigate the potential risk for animals and humans to be infected *via* the bites of infected ticks.
Materials & Methods: A total of 150 horses and 150 dogs used in Animal Assisted Interventions or living in contact with children, elderly people or immunocompromised persons were equally divided into clinically healthy animals and animals with at least one clinical compatible sign at the clinical examination or reported in the medical history. Sera were tested with ELISA and immunoblot for the presence of antibodies *vs* the target TBPs. The association between seropositivity and possible risk factors was statistically analyzed.
Results: Overall, 13 dogs (8.7%) and 19 horses (12.7%) were positive. One dog (0.7%) and 12 horses (8%), and 12 dogs (8.0%) and 10 horses (6.7%) were positive for *A. phagocytophilum* or *B. burgdorferi s.l.*, respectively. History of tick infestation in dogs was significantly associated with seropositivity ($p = 0.027$; OR 7.398).

Conclusions: These data show that in Italy ticks infected with *A. phagocytophilum* and *B. burgdorferi s.l.* circulate where dogs and horses are in contact with vulnerable people, and that these animals are useful epidemiological sentinels. The attention on these zoonotic diseases should be kept high, especially where vulnerable individuals are present and at risk of severe disease. Adequate routine serological monitoring and control plans for tick infestations in horse and dogs should be implemented to protect human and animal health.

P 034

Assessing temporal changes in microbial communities in *Hyalomma dromedarii* collected from camels in the UAE using high-throughput sequencing

N. Perveen[1], *S. B. Muzaffar*[1], *R. Vijayan*[1], *M. A. Al-Deeb*[1]
[1]United Arab Emirates University, Biology, Al Ain, United Arab Emirates

Ticks (Acari) are ectoparasites of animals that harbor communities of microbes of importance to animal and human health. Microbial communities associated with ticks exhibit temporal patterns of variation in their composition, with different genera dominating at different times of the year. In this study, molecular tools were used to assess the composition of the microbial communities associated with *Hyalomma dromedarii*. Adult ticks were collected every month for 1 year from 25 camels in the UAE. A total of 12 DNA pools were prepared (one pool for each month). We monitored the microbiota of ticks using high-throughput sequencing of the V3–V4 region of the bacterial 16S rRNA gene. A total of 614 operational taxonomic units were produced through de novo clustering and belonged to 17 phyla, 30 classes, 46 orders, 118 families, and 222 genera. Fifteen bacterial families were found to be the most abundant. The dominant bacterial communities associated with *H. dromedarii* belonged to the genera *Staphylococcus*, *Bacillus*, *Francisella*, and *Corynebacterium*, which were reported with high relative abundance from all months. No significant correlation occurred between the abundance of microbial families or genera in *H. dromedarii* ticks and the ambient temperature. Our findings revealed, for the first time in the UAE, temporal fluctuations of microbial communities in *H. dromedarii* ticks and provided key insights into the interaction between different microbial groups. Moreover, our results contribute to the current understanding of disease development and allow more investigations for potentially pathogenic microbiota.

P 035

Comparison of bacterial community between bisexual and parthenogenetic the Asian longhorn tick, *Haemaphysalis longicornis*

S. Hwang[1], *J. Kim*[1], *S. Y. Kim*[2], *H. I. Lee*[2], *D. Kim*[1]
[1]Kyungpook National University, Entomology, Sangju si, South Korea
[2]Korea Disease Control and Prevention Agency, Division of Vectors and Parasitic Diseases, Chungju, South Korea

The Asian longhorned tick, *Haemaphysalis longicornis*, is an obligate ectoparasite transmitting various pathogens including Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) and is widely distributed in East Asia including the Republic of Korea. *H. longicornis* reproduces offspring via either a bisexual or parthenogenesis reproductive system. It is hard to find morphological differences between two reproductive individuals, even though their fitness is quite different. In this study, we analyzed the partial sequences of 16s rDNA to identify the distribution pattern of bisexual and parthenogenesis individuals of *H. longicornis*, which were collected from twelve cities in the Rep. of Korea: Goseong[GS], Sokcho[SC], Ganghwa[GH], Samcheok[SCH], Sangju[SJ], Boryeong[BR], Ulsan[US], Gochang[GC], Jinju[JNJ], Jindo[JD], Jeju[JJ], and Seogwipo[SG]. Parthenogenesis individuals dominated the most areas in the mainland including Goseong, Ganghwa, Samcheok, and Gochang. Whereas bisexual individuals were dominant in southwestern regions including Jindo, Jeju, and Seogwipo. In addition, bacterial community was investigated to understand the interaction between microbes and reproductive system of *H. longicornis* by analyzing diversity, classification, and network of microbes in bisexual and parthenogenesis individuals. Taken together, parthenogenesis and bisexual *H. longicornis* individuals in Rep. of Korea were coexisted in the Rep. of Korea and bacterial community of two distinct reproductive systems was similar and/or different depending on the region and/or the system of reproduction. We expected that the present study would be helpful for a better understanding of the vectorial capacity of *H. longicornis.*

P 036

Neoehrlichia in small mammals and ticks from Hainich national park

L. Gothe[1], *C. Imholt*[2], *N. Król*[1], *M. Pfeffer*[1], *A. Obiegala*[1]
[1]University of Leipzig, Institute for Animal Hygiene and Veterinary Public Health, Leipzig, Germany
[2]Julius Kühn-Institute, Vertebrate Research, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Detection, Münster, Germany

The degree of land use intensity can be linked to the loss of ecosystem services, which is related to the loss of biodiversity in general. The mechanistic link between the loss of biodiversity and the increase in zoonotic pathogens is the disappearance of non-competent or dead-end hosts for pathogens. Losing these so-called non-reservoir-competent hosts enhances the transmission between competent hosts and vectors (termed “dilution effect”).

Neoehrlichia mikurensis (NM) is a tick-borne pathogen in Eurasia with rodents as natural reservoir hosts, which has not been studied for the effects of land use on its prevalence in ticks and reservoir hosts. NM is mainly transmitted by *Ixodes ricinus* and can cause severe disease associated with fever, rashes, and thromboembolic events, mostly in immunocompromised humans. Thus, this study aims to assess the prevalence of NM in questing ticks and rodents within a land-use gradient in the Hainich Dün region, Germany. Seasonal and multiannual fluctuations in NM prevalence in ticks and small mammals were studied within a closely monitored framework, including 25 woodland and 25 grassland spots.

In spring, summer, and autumn of 2020 and 2021, questing ticks were flagged and tested via real-time PCR targeting a partial fragment of the groEL gene to assess the prevalence of NM. Parallel to the tick collection, rodents were snap-trapped. Spleen samples of these rodents were tested via real-time PCR.

The overall prevalence of NM in the collected ticks was 3.9% (95% CI 3.11-4.86) and 9.1% (95% CI 8.22-9.99) in the rodent spleen samples.

NM is present in the studied tick and small mammal populations. Complete analysis of the data is still in progress. First results indicate a connection between land use intensities and infections with NM and support the dilution effect as a possible explanation for different habitat prevalences. These results show the need for evaluating the land use of a given environment and the prevalence of NM.

P 037

Molecular characterization of feline *Anaplasma phagoc*ytophilum strains by *ankA* gene and multilocus sequence typing

A. Kruppenbacher[1]*, I. Schäfer*[2]*, E. Müller*[2]*, F. D. von Loewenich*[1]
[1]University of Mainz, Institute of Virology, Mainz, Germany
[2]LABOKLIN GmbH and Co. KG, Bad Kissingen, Germany

Introduction: *Anaplasma phagocytophilum* is a Gram-negative obligate intracellular bacterium that replicates in neutrophil granulocytes. It is transmitted to humans and animals by ticks of the *Ixodes ricinus* complex. *A. phagocytophilum* causes febrile illness primarily in humans, in domestic animals such as dogs, horses and cats as well as in farm animals such as sheep, cattle and goats. In cats, granulocytic anaplasmosis has been described rarely. Further, only limited data regarding the *A. phagocytophilum* strains infecting cats exist.

Objectives: Therefore, genetic characterization was attempted in 8 feline *A. phagocytophilum* strains.

Materials & methods: DNA was isolated from the blood of 8 cats suffering from granulocytic anaplasmosis. Fragments of the *ankA* gene and seven housekeeping genes (*pheS, glyA, fumC, mdh, sucA, dnaN, atpA*) were amplified and sequenced bi-directionally. The sequences from the housekeeping genes were used for multilocus sequence typing (MLST).

Results: All gene fragments could be amplified from 5 samples. In the other 3 cases, the DNA was used up before completion. The phylogenetic analysis revealed that all *ankA* sequences belonged to the *ankA* cluster 1 that contains amongst others *A. phagocytophilum* strains from humans, dogs, and horses. In the MLST analysis, two sequence types (4 x ST 25, 1 x ST 188) were found. The two ST found belonged to the MLST cluster 1 that contains amongst other strains from humans, dogs, and horses.

Conclusion: Cats seem to be infected by the same *A. phagocytophilum* strains as humans, dogs, and horses. Therefore, the genetic characterization of their strains does not explain the fact that granulocytic anaplasmosis is much less frequently diagnosed in cats than in dogs and horses. It should be considered that the infection with *A. phagocytophilum* might be under-diagnosed in cats.

P 038

Detection of tick-borne encephalitis virus antibodies in sera of forestry workers, hunters and persons at risk in Mecklenburg-Western-Pomerania.

S. Frimmel[1], *T. Schwarz*[1], *G. Dobler*[2], *M. Löbermann*[1]
[1]University of Rostock Medical School, Infectious Diseases, Nephrology, Rostock, Germany
[2]Bundeswehr Institute of Microbiology, Microbiology, Munich, Germany

Question: Mecklenburg-Western-Pomerania, a federal state in the northeast of Germany has never been declared a risk area of TBE. A few autochthonous cases, along with TBEV-RNA detection in ticks, and detection of TBE-antibodies in sera of wild game and livestock have shown activity in natural foci of the virus in the past. To complement these data we examined sera of persons at risk for tick bites, forestry workers, hunters and people with increased tick exposure due to leisure activities.

Methods: The anti-TBE virus ELISA test kit "Vienna" from Euroimmun was used for screening. For differentiation of natural TBE infections and vaccination, NS1-ELISA was performed for all positive and borderline as well as negative controls. For confirmation of positive results serum neutralization test (NT) was performed.

Results: 274 subjects at risk for TBE were included for final analysis. 30% female 70% male. The average age 48.7 years (range 18-79). 43% of participants reported only recreational exposure to ticks, 21% were exposed in the workplace, 36% had exposure in both occupational and recreational settings. 138 subjects were screened positive, and 7 borderline, most of them vaccinated. Two subjects were also tested borderline in NS-1 ELISA and positive in neutralization test.

Conclusions: Two subjects can be discussed to have been infected autochthonously in Mecklenburg Western Pomerania, being tested borderline in the NS1-ELISA and positive with NT. One subject acquired the tick bites in the district of Ludwigslust-Parchim and one subject in the "Rostocker Heide", a recreational area near Rostock, formerly known as a natural focus of TBE, with occurrence of few autochthonous cases in the last decades. These results are an interesting complementation of data obtained from ticks, wild game, domestic animals and autochthonous cases to identify regions of possible risk for TBE-infection in Mecklenburg-Western-Pomerania.

This Project was funded by Pfizer Pharma GmbH.

P 039

New tick-borne encephalitis virus isolates from North-Eastern Germany and Western Poland

N. Król[1]*, L. Chitimia-Dobler*[2]*, G. Dobler*[2]*, D. Kiewra*[3]*, A. Czułowska*[3]*, A. Obiegala*[1]*, J. Zajkowska*[4]
T. Juretzek[5]*, M. Pfeffer*[1]
[1]University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany
[2]Bundeswehr Institute of Microbiology, Munchen, Germany
[3]University of Wrocław, Department of Microbial Ecology and Acaroentomology, Wrocław, Poland
[4]Medical University in Białystok, Department of Infectious Diseases and Neuroinfections, Białystok, Poland
[5]Carl-Thiem-Klinikum Cottbus gGmbH, Center for Laboratory Medicine, Microbiology and Hospital Hygiene, Cottbus, Germany

Tick-borne encephalitis virus (TBEV) belonging to the genus Flavivirus, family Flaviviridae, causes tick-borne encephalitis – medically the most important tick-borne viral disease. TBEV is transmitted to hosts mainly by ticks and is thought to circulate in very strictly defined natural foci. In Central Europe, so far only one of the five subtypes of TBEV has been found – the European subtype that is mainly associated with *Ixodes ricinus* but also detected in *Dermacentor reticulatus*. This study aims to identify new TBE foci in North-Eastern Germany and Western Poland.

Ticks were collected in areas indicated by TBE patients from Germany and Poland as places they were bitten by ticks and 19 potential TBE sites were chosen for further investigations. Ticks were collected from vegetation via the flagging method at selected locations. Sites were visited regularly from spring 2021 to summer 2022. Collected ticks were identified to life stage and species level and tested in pools for the presence of the TBEV using a real-time RT-PCR.

In total, 8400 ticks were collected at 19 sites. Four tick species were identified, dominant *I. ricinus* (n=4784; 56.9%), followed by *D. reticulatus* (n=3506; 41.7%), *Haemaphysalis concinna* (n=108; 1.3%), and *I. frontalis* (n=2; <0.1%). TBEV was detected in 19 tick pools with a MIR of 0.23% (95% CI: 0.14-0.36). The virus was found in: *I. ricinus* (13 pools; MIR=0.27%) and *D. reticulatus* (6 pools; MIR=0.17%). Positive samples originated in 6 locations – with the highest MIR of 1.05% and the lowest of 0.13%.

From 19 positive pools, 12 strains were sequenced and used for analyses – 10 from Germany and 2 from Poland. The phylogenetic analyses based on the E-gene revealed that strains from Saxony have the closest genetic relation to those from Baden-Württemberg and isolates from Brandenburg to strains from Finland and Estonia as well as Baden-Württemberg. Samples from Poland (Lower Silesia) cluster the closest with German strains.

P 040

MLST analysis of bird-related Lyme diseases borreliae in Japan

A. Takano[1], *S. Ishihara*[1], *A. Kawamura*[1], *K. Tatemoto*[2], *Y. Inoue*[1,2], *H. Shimoda*[1], *N. Nakamura*[3]
K. Maeda[1,2]
[1]Yamaguchi University, Yamaguchi, Japan
[2]National Institute of Infectious Diseases, Tokyo, Japan
[3]Yamashina Institute for Otnithology, Chiba, Japan

Question: Lyme borreliosis, a tick-borne disease caused by *Borrelia burgdorferi* sensu lato, is distributed in the northern hemisphere. In these pathogens, *Borrelia bavariensis*, *Borrelia garinii*, and *Borrelia afzelii* are the main species causing Lyme borreliosis in Asia.
It was reported that the reservoir animals of *B. bavariensis* and *B. afzelii* are mice, while *B. garinii* is maintained by birds. Therefore *B. garinii* has a wide geographical distribution and high genetic diversity. On the other hand, it is assumed that *B. bavariensis* has a narrow distribution range and low genetic diversity because it is carried by rodents. However, *B. bavariensis* distributed from Japan to Europe and these isolates are genetically indistinguishable. In this study, we tried to evaluate the contribution of birds in the expansion of *B. bavariensis* distribution.
Materials and Methods: We collected engorged larval ticks from wild birds captured from 2016 to 2018 and 2021 at 2 sites in Hokkaido. After molting to the nymph, we isolated the *Borrelia* spp. from ticks. After purification of genomic DNA from isolates, MLSA analysis was performed.
Results: In total, 372 strains were isolated. We detected 12 representative sequence types (STs) and 21 novel STs from bird-related strains. The 30 and 3 STs were classified as *B. garinii* and *B. bavariensis*, respectively. In this study, 5% (19/372) of isolates were identified as *B. bavariensis*.
Conclusions: In this study, *B. bavariensis* was isolated from bird-related ticks. Several strains were isolated each year. Therefore, it was suggested that *B. bavariensis* was maintained in birds at low rates. The wide distribution range of *B. bavariensis* may be related with the contribution of migratory birds.

P 041

Clade C aspartyl proteases in *Babesia divergens* and their validation as novel drug targets

P. Šnebergerová[1], *G. Lentini*[2], *O. Vadas*[2], *R. Visentin*[2], *L. Robbertse*[1], *J. Valdés*[1]
P. Bartošová-Sojková[1], *M. Jalovecká*[3], *D. Sojka*[1]
[1]Biology Centre of the Czech Academy of Sciences, Parasitology, České Budějovice, Czech Republic
[2]University of Geneva, Department of Microbiology and Molecular Medicine, Geneva, Switzerland
[3]University of South Bohemia, Faculty of Sciences, České Budějovice, Czech Republic

Introduction: Babesiosis is a tick-borne malaria-like disease caused by parasites from the apicomplexan genus *Babesia*. As in malaria-causing relatives, host cell invasion and egress are key processes for the propagation of *Babesia* intraerythrocytic stages. We identified two clade C *Babesia divergens* aspartyl proteases (BdASP3s), homologues of *Toxoplasma gondii* TgASP3 and *Plasmodium falciparum* PMIX/X, and evaluated their potential driving roles in proteolytic cascades and protein maturation associated with *Babesia* invasion and egress.

Objectives: Assessment of BdASP3 expression in *Babesia*. Evaluation of *B. divergens* susceptibility to 49c (the specific peptidomimetic inhibitor of TgASP3 and PMX/IX) ex vivo.

Performing in silico molecular docking of BdASP3s with 49c and assessing inhibitor specificity towards recombinant BdASP3s enzymes (rBdASP3s). Functional study of BdASP3s using transgenera complementation approach.

Materials and Methods: We produced rBdASP3s in two different expression systems. We used them to generate specific antibodies for immunostaining and microscopy and to evaluate their enzymatic activity and susceptibility to 49c.

Results: Here, we show the expression of native BdASP3s in *B. divergens* intraerythrocytic stages. 49c inhibited the propagation of *B. divergens* (ex vivo) and its specificity towards BdASP3s was evaluated in kinetic assays using rBdASP3s and fluorescent substrates. Multiplex substrate profiling of rBdASP3s also revealed the target cleavage sites and substrate preferences of these enzymes. Trans-genera complementation approach with iKD-TgASP3 *T. gondii* strain showed that BdASP3a mimics the secretory pathway of TgASP3 suggesting its involvement in protein maturation. However, BdASP3a did not subvert the iKD-TgASP3 deleterious phenotype indicating their species-specific function.

Conclusion: We demonstrate two BdASP3s as effectively druggable proteolytic targets for the development of the yet missing *Babesia*-specific chemotherapy.

P 042

Food-borne encephalitis and other modes of infection – a systematic review

E. Martello[1], *E. Gillingham*[2], *R. Phalkey*[2], *C. Vardavas*[3], *K. Nikitara*[3], *T. Bakonyi*[4], *C. M. Gossner*[4]
J. Leonardi-Bee[1]
[1]University of Nottingham, Division of Epidemiology and Public Health, School of Medicine, Nottingham, United Kingdom
[2]UK Health Security Agency, London, United Kingdom
[3]University of Crete, Medicine, Crete, Greece
[4]European Centre for Disease Prevention and Control (ECDC), Solna, Sweden

Introduction: Tick-borne encephalitis (TBE) is a viral infection caused by the tick-borne encephalitis virus (TBEv) and is common across parts of Europe. Transmission occurs mainly via tick bite (*Ixodes ricinus*, *Ixodes persulcatus*), but other non-vectorial modes of transmission are possible.

Objectives: This systematic review (SR) aims to collect and to synthesize the evidence on the non-vectorial modes of TBEv transmission in light of their epidemiological impact in Europe.

Materials & methods: Three electronic databases were searched for eligible studies (inception to April 2021). Further studies were identified from previous reviews and reference lists of included studies. The SR was conducted adhering to the JBI methodology for systematic reviews.

Results: A total of 41 studies were included, comprising of 1308 cases of TBE. Alimentary (36 studies), handling infected material (3 studies), blood-borne (1 study), and solid organ transplant (1 study) were identified as potential routes of TBEv transmission. We found no evidence of vertical transmission from mother to baby (2 studies), and there were no studies identified that assessed sexual or other non-vectorial routes of transmission. The most common vehicle of TBEv transmission was consumption of unpasteurised milk/milk products. Results of a random effects meta-analysis showed a significant three-fold increase in the risk of being positive to TBEv when consuming unpasteurised milk/milk products (pooled RR 3.05, 95% CI 1.53 to 6.11; 4 studies).
Conclusion: This review confirms alimentary as a route of TBEv transmission. In addition, handling infected material, blood-borne and solid organ transplant could be potential routes of TBEv transmission. Routine surveillance and awareness campaigns for both vectorial and non-vectorial transmission of TBEv together with promoting vaccination is important for the prevention and control of the disease, especially in high-risk areas.

P 043

Preliminary results from the burden of Lyme disease (BOLD) study – a prospective active surveillance study at primary care practices in endemic regions of six European countries

A. Loew-Baselli[1], *K. Halsby*[1], *A. Pilz*[1], *J. Stark*[2], *F. Angulo*[3], *J. S. Berglund*[4], *V. Cibik*[5], *L. Dybova*[6] *A. Moniuszko-Malinowska*[7], *J. Weimer*[8], *F. Strle*[9], *D. Zakova*[10], *J. Edwards*[2], *M. Bowdery*[11], *S. Valluri*[12] *B. J. Gessner*[2], *E. Begier*[13]
[1]Pfizer, Vienna, Austria
[2]Pfizer, Collegeville, PA, United States
[3]Pfizer, Portland, OR, United States
[4]Blekinge Institute of Technology, Karlskrona, Sweden
[5]Private Practice, Pruske, Slovakia
[6]Private Practice, Brno, Czech Republic
[7]Medical University of Bialystok, Bialystok, Poland
[8]Private Practice, Reinfeld, Germany
[9]University Medical Center Ljubljana, Ljubljana, Slovenia
[10]Private Practice, Trencin, Slovakia
[11]St. George's, University of London, London, United Kingdom
[12]Pfizer, New York, NY, United States
[13]Pfizer, Dublin, Ireland

Introduction: Lyme borreliosis (LB) is the most frequent tick-borne disease in Europe and the United States. Incidence estimates are highly variable. To prepare for a global efficacy trial for a candidate LB vaccine, we conducted prospective active surveillance at selected European primary care practices.
Objective: To assess LB incidence at sites in highly endemic regions in various European countries.
Methods: Preliminary data are presented from 13 practices in 6 countries (Czechia, Germany, Poland, Slovakia, Slovenia, Sweden). Suspected LB cases from each site's practice panel were recorded on screening logs and offered enrollment. Modified two-tiered testing using ELISAs was used to

identify laboratory-confirmed LB cases. All suspected cases were assigned a clinical diagnosis based on EUCALB-derived case definitions. Sites reported their practice panel size and historical LB case count by month (2019-20), allowing annualization of measured 2021 case counts and a projected 2021 LB incidence.

Results: The sites' mean duration of 2021 surveillance was 32 weeks (range: 23-38). During Apr 8–Dec 31 2021, sites identified 433 suspected LB cases; 351 (81%) were clinically diagnosed with LB. Projected LB incidence was 657/100,000 population in 2021, varying by and within country (Table). This was lower than in prior years based on historical LB counts except for Slovakia. 61% of clinically diagnosed cases were serologically-confirmed.

Conclusions: LB incidence was high and variable across sites and countries in 2021, highlighting the need rigorous ascertainment methods. The proportion of serologically-confirmed cases is aligned with the expectation that about half of erythema migrans cases identified would seroconvert. Culture and PCR testing of skin biopsy specimens will likely result in a higher proportion of laboratory confirmed LB cases in the final results. The BOLD study is continuing through 2022.

P 044

Clinical data on reinfections with *Borrelia burgdorferi* sensu lato

V. Maraspin[1], S. Lotrič-Furlan[1], J. Cimperman[1], E. Ružić-Sabljić[2], K. Ogrinc[1], T. Rojko[1], P. Bogovič[1] K. Strle[3], F. Strle[1]
[1]University Medical Center Ljubljana, Ljubljana, Slovenia
[2]University of Ljubljana, Microbiology and Immunology, Ljubljana, Slovenia
[3]Wadsworth Center, NYS Department of Health, New York, NY, United States

Introduction: Current information on clinically manifested reinfections with *B. burgdorferi* s.l. is limited to individual case reports and a few small case series [SK1] which suggest that erythema migrans (EM) is almost exclusive clinical manifestation of the initial and repeated infection.

Objectives: To assess if reinfections occurred after other manifestations of Lyme borreliosis (LB), and to evaluate if different manifestations of LB provided distinct protection against clinically manifested reinfection.

Methods: Our study is based on prospectively collected data in 11,642 adults, diagnosed with typical EM at LB Outpatient's Clinic (LBOC) Ljubljana during 1990–2014. The dataset included history of LB within 10 years before current EM.

Results: Of 11,642 patients identified with EM in the 25-year period, 1215 (10.4%) had been diagnosed and treated for a previous episode of LB 2 months to 9 years (median 3.5 years) before the visit to our LBOC. The most frequent previous episode of LB was EM (1102/1215, 90.7%). However, 60 (4.9%) cases had other manifestation of LB: 37 Lyme neuroborreliosis, 10 Lyme arthritis, 12 chronic atrophic acrodermatitis, and 1 borrelial lymphocytoma. Moreover, 53 (4.4%) patients without clinically evidenced LB were treated for unspecific symptoms and positive borrelial serum antibodies. [SK2] The subgroup of 60 patients with previous manifestation of LB other than EM had 3-times lower odds for presenting to our LBOC with multiple EM than patients with antecedent episode of EM, while in those who had had unspecific symptoms compounded with positive borrelial serum antibodies the odds were similar as in patients with previous EM.

Conclusions: Previous episode of LB was reported in 10% of our patients with EM. Clinically manifested reinfection is relatively common after EM. Reinfection may occur after other manifestations of LB, however with 3-fold lower risk of dissemination, implying that the immune response is playing a protective role in this disease.

P 045

Molecular detection of ricketsia in ticks collected from big-eared opossum (*Didelphis aurita*) in forest parks in the metropolitan region of São Paulo, Brazil

M. P. D. Albuquerque[1,2], *R. Antonio Silva*[1], *A. Pinter*[1,2], *T. Fernandes Martins*[1,3]
[1] Pasteur Institute, São Paulo, Brazil
[2]University of São Paulo, Faculdade de Saúde Pública, São Paulo, Brazil
[3]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Departamento de Medicina Veterinária Preventiva e Saúde Animal, São Paulo, Brazil

Big-eared opossums (*Didelphis aurita*) are marsupials considered synanthropic and reservoirs of zoonotic agents of public health importance. During a study on the health aspects of *D. aurita* and its relationship with Chagas disease and triatomines, ticks were also collected from these wild marsupials. The animals were captured in nine forest parks located in the metropolitan region of São Paulo. In order to detect rickettsia in opossum ticks, they were inspected for the presence of ectoparasites during captures between March 2020 and April 2022. The ticks collected from opossums were identified using a stereomicroscope and taxonomic keys for ticks, and molecularly tested for the presence of rickettsiae using *omp*A and *glt*A genetic markers The forest parks sampled, with the 67 parasitized opossums and the species of ticks identified were: *Ixodes loricatus* (88); *Amblyomma dubitatum* (10) nymphs; *Amblyomma sculptum* (2) and *Amblyomma* sp. (44). In the present study, *Rickettsia belli* was detected in *I. loricatus*, a rickettsia associated with ticks, but of unknown pathogenicity for humans. We demonstrated that opossums are important hosts for ticks in all parasitic stages (larvae, nymphs and adults). Here we find adults of *A. sculptum* parasitizing opossums, being considered the main vector of Brazilian spotted fever and the most aggressive tick for humans in the national territory. Our study highlights the need for more research on rickettsiae, given the frequent human visits in these forest parks, the proximity of opossums to humans, and the occurrence of the vector in the metropolitan region of São Paulo, Brazil.

P 046

Characterization of tick species and tick-transmitted protozoa and bacteria in wild ruminants in a French zoological park

M. Jouglin[1], H. Duchêne[1], B. Blanc[2], A. Brunet[2], N. de la Cotte[3], C. Bonsergent[1], A. Agoulon[3]
S. Bastian[3], K. Ortiz[2], L. Malandrin[1]
[1]INRAE, UMR BIOEPAR, Nantes, France
[2]Muséum National d'Histoire Naturelle, Zoology, Obterre, France
[3]Oniris, UMR BIOEPAR, Nantes, France

Introduction: Ticks and tick-transmitted diseases are seldomly studied in the context of a concentrated, multi-specific collection of mammals preserved in a forested area.
Objectives: Symptoms of hyperthermia with severe apathy were observed in several cervids recently arrived at the Réserve Zoologique de la Haute Touche (Center of France). A longitudinal survey of the ticks and tick-borne microbes present was undertaken to investigate the exposure of the animals to tick-borne pathogens.
Materials and methods: Questing ticks were collected using the white flag method, on more than 100 sampling points, twice in late spring and once in the fall. Blood samples were collected from 828 animals, from 64 species, most of them ungulates, over a period of 4 years. Nested PCRs and sequencing were performed to detect and characterize piroplasms (*Babesia* spp. and *Theileria* spp.), *Anaplasma* spp. and *Bartonella* spp.
Results: Three main tick species were identified: *Ixodes ricinus*, *Haemaphysalis concinna* and *Haemaphysalis inermis*. We found that 21.1 % of animals tested were PCR-positive for the protozoan genus *Theileria*, 9.8 % for *Bartonella* spp., and 13.1% for *Anaplasma* spp. Cervids were significantly more frequently infected compared to bovids. *Anaplasma phagocytophilum* was identified and for the first time in Europe, *A. capra* was characterized, in swamp deer and red deer. We also subtyped *Bartonella* spp. to *B. bovis*, *B. chomelii* and *B. schoenbuchensis*. Different *Theileria* genotypes were detected.
Conclusion: This study shows that a high diversity of ticks and tick-transmitted microbes are endemic in this multi-specific collection of mammals. The findings will enrich our use of laboratory diagnosis, for understanding the etiology behind these non-specific symptoms and designing prevention strategies.

P 047

A metagenomic sequencing panel using nanopore reveals circulating pathogens in UK wildlife

M. White[1], *S. Pullan*[2], *R. Vipond*[2], *J. Medlock*[3], *E. Feil*[4]
[1]UK Health Security Agency, Medical Entomology and Zoonoses Ecology/Genomics, Salisbury, United Kingdom
[2]UK Health Security Agency, Genomics, Salisbury, United Kingdom
[3]UK Health Security Agency, Medical Entomology and Zoonoses Ecology, Salisbury, United Kingdom
[4]University of Bath, The Milner Centre of Evolution, Claverton down, Bath, United Kingdom

Game animals such as deer and pheasants are abundant in the UK. Ticks frequently feed on these animals, yet little is known about their role in tick pathogen cycles despite their close contact with humans. A metagenomic nanopore-based sequencing panel has been developed for ticks to analyse several targets in ticks, identify the source of their last blood meal, species of tick, characterisation of the bacterial microbiome, and to speciate *Borrelia.* This sequencing panel was used on ticks collected from both game animals and other wildlife found in the UK. *Borrlia afzelii* and *B. garinii* are known to be associated with birds and have previously been detected in pheasants in the UK. However, it is currently unknown whether there are any other species or potential bacterial pathogens present in game birds. Ticks at both the larval and nymphal stage feed on ground-nesting birds, so it can be assumed that there is some contribution to pathogen prevalence. Deer have been widely studied in their role as dilution hosts for *Borrelia* and are known hosts of *Anaplasma* in the UK. This study aims to investigate the bacterial microbiome of ticks fed on wildlife and game animals, as well as pathogen prevalence. The findings presented here could provide new insights into important reservoir hosts of tick-borne disease and the role of the microbiome in disease transmission.

P 048

Asymptomatic carriers of equine piroplasmosis – prevalence differences between 4 French regions and low genetic diversity of *Theileria equi* and *Babesia caballi*

M. Jouglin[1], F. Berquier[1], C. Bonsergent[1], N. de la Cotte[2], C. Bizon[3], A. Bohec[4], A. Courouçé[3] O. Guibert[4], E. Lallemand[5], L. Lemonnier[3], A. Leblond[4], A. Leroux[3], I. Marano[5], A. Muzard[3], A. Prévôt[4], E. Quéré[6], M. Thirouin[4], M. Toussaint[5], A. Agoulon[2], L. Malandrin[1]
[1]INRAE, UMR BIOEPAR, Nantes, France
[2]Oniris, UMR BIOEPAR, Nantes, France
[3]Oniris, CISCO, Nantes, France
[4]VetAgro Sup, Lyon, France
[5]Ecole Nationale Vétérianire de Toulouse, Toulouse, France
[6]Ecole Nationale Vétérinaire de Maisons-Alfort, Maisons-Alfort, France

Introduction: Equine piroplasmosis is a tick-borne disease of equids, and is caused by two protozoan parasites, *Theileria equi* and *Babesia caballi*.
Objectives: The aim of our study is to evaluate the prevalence of carrier horses in France and to determine the genetic diversity of both *T. equi* and *B. caballi*.
Materials and methods: Blood samples were collected between 2019 and 2022 from horses without any symptoms of piroplasmosis. The equids were presented in each of the 4 French Veterinary Schools in France located in Maisons-Alfort (North central, 106 equids), Nantes (West central, 165 equids), Lyon (South East, 151 equids) and Toulouse (South West, 75 equids). Each school was considered as a focal point with horses located in their surroundings.
The infection status of equids was determined by nPCR specific for each parasite, targeting the 18S rRNA gene, and genotyping was performed by sequencing partial 18S rRNA.
Results: As a whole, 40.5% of the equids were asymptomatic carriers, with a majority of *T. equi* carriers (38.8%) and few *B. caballi* carriers (3.9%).
The piroplasmosis infection prevalence varied greatly between the four veterinary schools with an increasing North to South gradient: 16% around Paris, 30.3% around Nantes, 53.6% around Lyon, and 72.9% around Toulouse.
Genotyping was performed on 193 *T. equi*-positive samples and on 18 *B. caballi* sequences. BLASTn and phylogenetic analysis revealed that 98% of *T. equi* sequences belonged to the genotype E, previously described in several European countries. The remaining isolates belonged to the A genotype, described in many countries around the world. All 18 *B. caballi* sequences clustered in the A genotype.
Conclusion: We demonstrate a high prevalence of *T. equi* carrier horses in France with a North to South increasing gradient. Prevalence of *B. caballi* was found to be low. The genetic diversity of both parasites was found to be low in France.

P 049

Tracking the emergence of ticks and tick-borne diseases through community-engaged tick surveillance

S. Thangamani[1], *B. Andrews*[1], *A. Hodge*[1], *E. Reynolds*[1], *C. Hart*[1]
[1]SUNY Upstate Medical University, Microbiology and Immunology, Syracuse, NY, United States

Ticks and tick-borne diseases continue to emerge in the Northeastern United States. To track the emergence of ticks and tick-borne pathogens, we started a community-engaged passive surveillance program (www.nyticks.org). Ticks encountered by humans are sent to our lab for identification and pathogen testing. Before sending the ticks, the public provides information such as the date and place of the tick encounter through an online tick submission form. In the lab, we morphologically identify ticks and process the samples to detect 16 tick-borne agents commonly present in the United States. Based on our data, *I. scapularis* is the most common tick encountered by humans, and they are infected with pathogens of human relevance, including *B. burgdorferi*, *B. miyamotoi*, *A. phagocytophilum*, *B. microti*, and Powassan virus. In addition, the geographic and temporal distribution of tick species and pathogens was determined. Data collected from the public and in the lab enabled us to construct a powerful visual analytical mapping tool, *tick*MAP, to track the emergence of ticks and tick-borne pathogens in real time. The public can use this tool to identify hot spots of disease emergence, clinicians for supportive evidence during differential diagnosis, and researchers to better understand factors influencing the emergence of ticks and tick-borne diseases in New York. Overall, we have created a community-engaged tick surveillance program and an interactive visual analytical *tick*MAP that other regions could emulate to provide real-time tracking and an early warning for the emergence of tick-borne diseases.

P 050

Does it work? effects of permethrin-treated uniforms on tick submissions to a military passive tick surveillance program

R. M. Nadolny[1], *J. Jones*[1], *B. J. Taylor*[2], *L. Bowman*[1], *K. Harkins*[3]
[1]Defense Centers for Public Health-Aberdeen, Vector-Borne Disease Branch, Laboratory Sciences Directorate, Aberdeen Proving Ground, MD, United States
[2]Defense Centers for Public Health-Aberdeen, Office of Public Health Scientific Integrity, Aberdeen Proving Ground, MD, United States
[3]Defense Centers for Public Health-Aberdeen, Entomological Sciences Division, Environmental Health Sciences and Engineering Directorate, Aberdeen Proving Ground, MD, United States

Introduction: Permethrin-treated clothing is often recommended as a first line of defense against tick-borne diseases. In 2012, the permethrin factory-treated Army Combat Uniform (ACU) was first made available to United States Active Duty Soldiers, Army National Guard, Army Reserve Enlisted Soldiers, and the Senior/Junior Reserve Officers Training Corps (ROTC). Subsequently, Air Force and Marine personnel were also offered permethrin factory-treated uniforms.

Objectives: Here, we use the passive surveillance data collected through the Military Tick Identification/ Infection Confirmation Kit (MilTICK) program to determine the effectiveness of permethrin-treated uniforms at protecting Department of Defense (DoD) personnel from exposure to tick-borne pathogens in the United States.
Materials and Methods: We analyzed whether submissions by self-reported users of the permethrin-treated uniforms had smaller proportions of engorged ticks, certain cohorts of MilTICK users were better protected than others, and specific tick species were better repelled than others.
Results: We found that permethrin use resulted in significantly lower proportions of engorged ticks submitted across MilTICK users. In the presence of permethrin, service member groups experienced lower proportions of ticks feeding to engorgement compared to civilians, dependents, and retirees. For submissions from active duty personnel serving in uniform, the proportion of tick engorgement in the presence of permethrin increased over time, possibly indicating that permethrin-treated uniforms suffer from reduced performance over the life of the garment. We also found that while permethrin reduced all tick species” ability to feed until engorgement, blacklegged ticks and lone star ticks were better able to resist permethrin”s lethal effects than American dog ticks.
Conclusion: Permethrin treated clothing plays an important role in keeping military personnel safe from tick-borne diseases.

P 051

Ornithological and molecular evidence of a reproducing *Hyalomma rufipes* population under continental climate in Europe

G. Keve[1,2], *T. Csörgő*[3,4,5], *A. Benke*[5,6], *A. Huber*[5,7], *A. Mórocz*[5,8], *Á. Németh*[5,9,10], *B. Kalocsa*[5]
E. A. Tamás[5,11], *J. Gyurácz*[5,12], *O. Kiss*[5,13], *D. Kováts*[4,5,14], *A. D. Sándor*[1,2,15], *Z. Karcza*[5,15], *S. Hornok*[1,2]
[1]University of Veterinary Medicine, Department of Parasitology and Zoology, Budapest, Hungary
[2]ELKH-ÁTE Climate Change: New Blood-sucking Parasites and Vector-borne Pathogens Research Group, Budapest, Hungary
[3]Eötvös Loránd University, Department of Anatomy, Cell- and Developmental Biology, Budapest, Hungary
[4]Ócsa Bird Ringing Station, Ócsa, Hungary
[5]BirdLife Hungary, Budapest, Hungary
[6]Fenékpuszta Bird Ringing Station, Fenékpuszt, Hungary
[7]Aggtelek National Park Directorate, Jósvafő, Hungary
[8]Duna-Dráva National Park Directorate, Pécs, Hungary
[9]Kiskunság National Park Directorate, Kecskemét, Hungary
[10]Kiskunság Bird Protection Association, Izsák, Hungary
[11]University of Public Service, Faculty of Water Sciences, Baja, Hungary
[12]Eötvös Loránd University, Biology, Szombathely, Hungary
[13]University of Szeged, Faculty of Agriculture, Institute of Animal Sciences and Wildlife Management, Hódmezővásárhely, Hungary
[14]Hungarian Biodiversity Research Society, Budapest, Hungary
[15]University of Agricultural Sciences and Veterinary Medicine, Department of Parasitology and Parasitic Diseases, Cluj-Napoca, Romania

Introduction: Although the role of migratory birds in carrying ticks is known from all parts of Europe, in most countries no contemporaneous multiregional surveillance of bird-associated ticks was reported.
Objectives: Investigation of the role of birds in the epidemiology of ticks.
Materials & Methods: Ticks were collected from birds at seven ringing stations in Hungary, in 2022. *Hyalomma* species were identified molecularly. Morphological methods were used for other genera.
Results: From 38 passeriform bird species 956 ixodid ticks were collected. Tick species were identified as *Ixodes ricinus* (n=598), *Ixodes frontalis* (n=18), *Ixodes lividus* (n=6), *Haemaphysalis concinna*(n=321) and *D. reticulatus* (n=1). All 12 *Hyalomma* sp. ticks (11 engorged nymphs and 1 unengorged larva) were identified as *H. rufipes* based on three mitochondrial markers. This species was only found in the Transdanubian region and along its southeastern border. The Common Blackbird and the European Robin were the two main hosts of *I. ricinus* and *I. frontalis*, whereas *H. concinna* was almost exclusively collected form long-distance migrants. The predominant hosts of *H. rufipes* were reed-associated bird species, the Sedge Warbler and the Bearded Reedling, both in their nesting period.
Conclusions: This study provides an explanation for the over-century-long presence of adult *Hyalomma* ticks in the Transdanubian Region of the Carpathian Basin. More importantly, the autochthonous occurrence of a *H. rufipes* population was revealed for the first time in Europe, based on the following observations: (1) none of the bird species infested with *H. rufipes* are known to migrate during

their nesting period; (2) one larva was not yet engorged; (3) the larva and the nymphs must have belonged to different local generations; and (4) all *H. rufipes* found in the relevant location were identical in their haplotypes. This study also demonstrated regional and temporal differences in tick species carried by birds.

P 052

Infection of free-living ungulates and feeding ticks from south-western Slovakia with Piroplasmida and *Anaplasma phagocytophilum*

D. Selyemová[1], *M. Profotová*[1], *L. Anettová*[1], *S. Purgatová*[2], *Z. Krumpálová*[2], *M. Kazimírová*[1]
[1]Slovak Academy of Sciences, Zoology, Bratislava, Slovakia
[2]Constantine the Philosopher University, Department of Ecology and Environmental Studies, Faculty of Natural Sciences and Informatics, Nitra, Slovakia

Wildlife are important reservoirs of zoonotic Piroplasmida (*Babesia* spp., *Cytauxzoon* spp., *Theileria* spp.) and *Anaplasma phagocytophilum*.
In Slovakia, data on the reservoir role of wildlife for zoonotic *Babesia* spp. and *A. phagocytophilum* strains is limited. This study is aimed at molecular identification of Piroplasmida and *A. phagocytophilum* in tissue samples of wild ungulates and ticks infesting them. Spleen samples and engorged ticks were collected from hunted cervids, mouflons and wild boar from south-western Slovakia (regions Bratislava, Trnava, Záhorie, Levice) during 2018-2022. *Babesia/Theileria* were detected by PCR targeting a 450 bp fragment of the 18S rRNA gene, *A. phagocytophilum* by real-time PCR targeting the *msp2* gene. Positive amplicons for *Babesia/Theileria* were sequenced. In Bratislava, Trnava and Záhorie regions, ungulates were infested with *Ixodes ricinus*, *Haemaphysalis concinna* and *Dermacentor reticulatus*. Cervids (44-100%) were positive for *Theileria capreoli,* which also prevailed in *I. ricinus* and *H. concinna*. *B. venatorum* was detected in one *I. ricinus* from roe deer. *Babesia* spp. were detected in *D. reticulatus* and *I. ricinus* from wild boar. *A. phagocytophilum* was present in 79-90% cervids and 31% wild boars. In Levice, sympatric occurrence of five tick species (*I. ricinus*, *D. reticulatus*, *D. marginatus*, *H. concinna*, *H. inermis*) was recorded. Red deer were infested with *I. ricinus*, *H. concinna* and *H. inermis*. From wild boar, all five tick species were collected. *T. capreoli* was found in 85% red deer. *Babesia* sp. was detected in one wild boar (6%). *T. capreoli* was detected in *I. ricinus*, *H. inermis* and *H. concinna* from cervids. *Babesia* spp. were detected in *H. concinna* and *D. reticulatus* from wild boar. The low prevalence of *B. venatorum* in ticks and its absence in ungulates suggests that the risk of contracting zoonotic *Babesia* in the studied regions is low.
Acknowledgements. The work was funded by grants KEGA 1/019UKF-4/21 and VEGA 2/0137/21.

P 053

Fitness of mCherry reporter tick-borne encephalitis virus in tick experimental models

A. Kevély[1,2], *V. Prančlová*[3,4], *M. Sláviková*[1], *J. Haviernik*[4,5,6], *V. Hönig*[4,5], *E. Nováková*[1,2], *M. Palus*[4,5]
D. Růžek[4,5,6], *B. Klempa*[1,2], *J. Koči*[1,7]
[1]Slovak Academy of Sciences, Institute of Virology, Biomedical Research Center, Bratislava, Slovakia
[2]Comenius University in Bratislava, Department of Microbiology and Virology, Faculty of Natural Sciences, Bratislava, Slovakia
[3]University of South Bohemia, Faculty of Sciences, Ceske Budejovice, Czech Republic
[4]Czech Academy of Sciences, Institute of Parasitology, Biology Centre, Ceske Budejovice, Czech Republic
[5]Veterinary Research Institute, Brno, Czech Republic
[6]Masaryk University, Department of Experimental Biology, Faculty of Science, Brno, Czech Republic
[7]Slovak Academy of Sciences, Zoology, Bratislava, Slovakia

The tick-borne encephalitis virus (TBEV) causes a most important viral life-threatening illness transmitted by ticks. The interactions between the virus and ticks are largely unexplored, indicating a lack of experimental tools and systematic studies. One such tool is recombinant reporter TBEV, offering antibody-free visualization to facilitate studies of transmission and interactions between a tick vector and a virus. In this paper, we utilized a recently developed recombinant TBEV expressing the reporter gene mCherry to study its fitness in various tick-derived *in vitro* cell cultures and live unfed nymphal *Ixodes ricinus* ticks. The reporter virus was successfully replicated in tick cell lines and live ticks as confirmed by the plaque assay and the mCherry-specific polymerase chain reaction. Although a strong mCherry signal determined by fluorescence microscopy was detected in several tick cell lines, the fluorescence of the reporter was not observed in the live ticks, corroborated also by immunoblotting. Our data indicate that the mCherry reporter TBEV might be an excellent tool for studying TBEV-tick interactions using a tick *in vitro* model. However, physiological attributes of a live tick, likely contributing to the inactivity of the reporter, warrant further development of reporter-tagged viruses to study TBEV in ticks *in vivo*.

Acknowledgment: This research was funded by the grants H2020 project# 101030179-TVISTOFF, TBFVnet: surveillance and research on tick-borne flaviviruses (TBFV) 2018-1-0659, APVV-18-0201 and APVV-21-0431 from the Slovak Research and Development Agency, and 20-14325S and 20-30500S from the Czech Science Foundation. J.K. is a recipient of Marie Sklodowska-Curie reintegration fellowship, H2020-MSCA-IF-2020, project# 101030179-TVISTOFF.

P 054

Co-infection with multiple strains of *Borrelia burgdorferi* in female mice reduces maternal antibody protection in their offspring

A. Foley-Eby[1], *E. Uhlemann*[2], *G. Hurry*[2], *M. Voordouw*[1]
[1]University of Saskatchewan (WCVM), Veterinary Microbiology, Saskatoon, Canada
[2]University of Saskatchewan, Saskatoon, Canada

Vertebrate hosts are often co-infected with multiple strains of *Borrelia burgdorferi* (*Bb*). Females infected with *Bb* can transmit maternal antibodies to their offspring during pregnancy and lactation, which can protect against infectious challenge. The purpose of this study was to investigate whether co-infection in the mother reduces the protective efficacy of the maternal antibody response in her offspring. Strains of *Bb* express different variants of outer surface protein C (OspC), an immunogenic antigen associated with strain-specific immunity. This study used two strains of *Bb* that express OspC types A and I (hereafter strain A and strain I). Female mice were assigned to 4 treatments (8 mice per treatment): (1) infected with strain A, (2) infected with strain I, (3) co-infected with both strains, and (4) uninfected. Female mice were paired with male mice at 3 weeks post-infection to produce offspring. At 4 weeks post-birth (PB), 88 offspring were confirmed to be uninfected with *Bb,* but their blood contained maternal antibodies that were specific for the two different OspC types. Offspring were challenged with both strains via tick bite at either 6 weeks PB ($n = 45$) or 11 weeks PB ($n = 43$). For offspring from mothers infected with a single strain, maternal antibodies provided almost perfect protection against the homologous (but not heterologous) strain at both 6 and 11 weeks PB. In contrast, for offspring from co-infected mothers, the rate of break-through infections was ~50% at 6 weeks and 100% at 11 weeks. All break-through infections were caused by strain A and never strain I. Level of IgG antibodies specific for OspC types A and I were considerably lower in offspring from co-infected mothers compared to offspring from mothers with single strain infections. Our study demonstrates that co-infection in the mother reduces the protective efficacy of the maternal antibody response in her offspring.

O 025

Cerebrospinal fluid involvement in a Murine model of Lyme neuroborreliosis

C. Brissette[1]
[1]University of North Dakota, School of Medicine and Health Sciences, Department of Biomedical Sciences, Grand Forks, ND, United States

The cerebrospinal fluid (CSF) is an important signaling medium between the meninges and the brain, and Lyme neuroborreliosis patients often present with inflammatory cytokines/chemokines, lymphocytic pleocytosis, *Borrelia*-specific antibodies, and live spirochetes in the CSF. We previously demonstrated that *Borrelia burgdorferi* routinely colonizes the dura mater of laboratory mice, with persistent bacterial colonization, vascular damage, leukocyte infiltration, and increased inflammatory markers. The brain parenchyma of these mice displays a distinct Type I interferon (IFN) response in the absence of spirochete colonization. In the current study, we investigate the role of the CSF in perpetrating the signal of *B. burgdorferi* colonization of the meninges to the brain. We show that *B. burgdorferi* readily colonizes the CSF with high detection sensitivity, associated with increased cytokine levels. CXCL13 and IL-6 were most predominant CSF cytokines. Increased Type 1 IFN was specific to the CSF, whereas Type 2 IFN was specific to serum. Fluid-specific cytokine differences may be due to differences in the site of production or differences in the ability of different cytokines to enter the CSF from the periphery. Further characterization of the CSF response will include the production of intrathecal *B. burgdorferi*-specific antibodies.

O 026

Clinically manifested reinfections with *Borrelia burgdorferi* sensu lato – Comparison of erythema migrans during primary infection and reinfection

F. Strle[1], *V. Maraspin*[1], *S. Lotrič-Furlan*[1], *J. Cimperman*[1], *E. Ružić-Sabljić*[2], *T. Rojko*[1], *K. Ogrinc*[1]
K. Strle[3], *P. Bogovič*[1]
[1]University Medical Center Ljubljana, Ljubljana, Slovenia
[2]University of Ljubljana, Microbiology and Immunology, Ljubljana, Slovenia
[3]Wadsworth Center, NYS Department of Health, New York, NY, United States

Introduction: Information on clinically manifested reinfections with *Borrelia burgdorferi* s.l. is limited. No reliable information on the course of primary and subsequent erythema migrans (EM) exists.
Objectives: To ascertain differences in EM between primary infection and reinfection.
Methods: The study is based on prospectively collected clinical information on 12,384 adult patients with typical EM diagnosed at the Lyme borreliosis Outpatient's Clinic (LBOC) in Ljubljana from 1990 to 2014. We compared findings in patients who had EM for the first time with those in whom EM was the result of reinfection.
Results: Of 12,384 EM patients in the database 1215 patients were excluded because they had a previous episode of LB diagnosed elsewhere. Thus, in total, data from 11,169 were evaluated in this study, 10,534 EM patients without previous LB and 635 patients with preceding EM. Patients with

reinfection were older (56 versus 48 years; $p<0.0001$), were more frequently females (66.5% versus 63.7%; $p=0.0031$), less often developed multiple EM (4.4% versus 7.3%; $p<0.0001$; $OR=0.59$, 95% CI: 0.39–0.87) and less often reported symptoms at the site of EM skin lesion (43.3% versus 53.6%; $p<0.0001$). In contrast both groups had a similar frequency of constitutional symptoms (34.4% versus 34.8%; $p=0.882$) and a similar isolation rate of *B. burgdorferi* s.l. from skin (257/520, 49.4% versus 3592/7180, 50.0%; $p=0.825$). Reinfection was significantly more common in patients having underlying immunocompromised condition (6.8% versus 2.4%; $p<0.0001$; OR 3.04, 95% CI: 2.14–4.29).

Conclusions: Several demographic and clinical characteristics distinguish EM due to primary infection or reinfection. One of the main risk factors for reinfection is immunodeficiency of the host. Coupled with decreased lower frequency of multiple EM in reinfected individuals, these data suggest some level of immunological memory may prevent reinfection in immunocompetent host.

O 027

Transcriptional regulation of the essential *ospC* gene of the Lyme disease spirochete

B. Stevenson[1]
[1]University of Kentucky, Microbiology, Immunology, and Molecular Genetics, Lexington, KY, United States

The OspC outer surface protein is essential for initial stages of vertebrate infection by *Borrelia burgdorferi* sensu lato. The *ospC* gene is not expressed by bacteria within unfed ticks. As ticks feed, OspC is induced, then expressed for the first 1-2 weeks of vertebrate infection, after which *OspC* is repressed. Bacteria that either fail to express OspC early, or fail to repress OspC later, are rapidly cleared by host immune responses.

Using DNA adjacent to the *ospC* promoter as bait, DNA affinity studies identified a protein named Gac, which is produced from the C-terminal half of the *gyrA* gene (Gac = GyrA C-terminus). We found that Gac binds to a repeated 11-bp sequence adjacent to the *ospC* promoter. Further investigations revealed that Gac is a transcriptional repressor of *ospC*.

Subsequent affinity studies identified two more proteins that bind to *ospC* 5’ DNA: DnaA and BpuR. We found that the *ospC* operator (5’ of the promoter) is required to prevent repression by Gac, suggesting that DnaA and/or BpuR de-repress *ospC*by antagonizing Gac binding.

The complex tick-vertebrate-tick infectious cycle of *B. burgdorferi* requires numerous interactions between the bacteria and vector/host tissues. However, this cycle never varies to any significant extent. As a result, we hypothesize that the Lyme disease spirochete has evolved to identify conditions that are unique to each step of the cycle, and thereby “know” where it is and what response is needed. Our previous studies found that *B. burgdorferi* induces expression of OspC following increases in bacterial replication rate. The only point in its infectious cycle at which bacterial replication increases substantially is when a tick ingests nutritious blood. This permits the spirochete to associate increased replication rate with tick feeding, and respond by up-regulating OspC and other virulence factors that are required during transmission and early in infection. Hence the involvement of DnaA in coordinating those processes.

O 028

Versatile roles of PFam54 orthologs in complement inactivation of serum resistant *Borrelia bavariensis* isolates from Japan

L. Langhoff[1], P. Kapfer[1], J. Wülbern[2], F. Röttgerding[1], M. Nakao[3], K. Sato[4], H. Kawabata[4]
R. E. Rollins[5], P. Kraiczy[1]
[1]University Hospital Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt a.M., Germany
[2]Christian-Albrechts-Universität zu Kiel, Biology, Kiel, Germany
[3]Asahikawa Medical University, Parasitology, Asahikawa, Germany
[4]National Institute of Infectious Diseases, Bacteriology, Tokyo, Japan
[5]Institute of Avian Research, Wilhelmshaven, Germany

Borrelia (B.) bavariensis, a causative agent of Lyme borreliosis in Europe and Asia is associated with severe neurological manifestations. To overcome complement, European *B. bavariensis* isolates possess at least two distinct immune evasion molecules, BGA66 and BGA71. So far, only fragmentary information is available on the complement inhibitory activity of orthologous proteins derived from Asian *B. bavariensis* isolates. Here, we investigated serum resistance of NT24 and JHM1114, two Japanese *B. bavariensis* isolates and assessed the complement inhibitory activity of distinct orthologs. Whole genome sequencing showed that NT24 contains BGA66 and BGA71 including a novel ortholog, termed BGA68b. However, JHM1114 lacks both BGA66 and BGA71 but contains two novel orthologs, termed BGA67b and BGA71b.

Employing serum bactericidal assays, NT24 and JHM1114 resist complement-mediated killing in human serum. To further assess complement inhibitory activity, BGA66$_{NT24}$, BGA68$_{NT24}$, BGA71$_{NT24}$, BGA67b$_{JHM1114}$, and BGA71b$_{JHM1114}$ were investigated. Our functional analyses showed that BGA66$_{NT24}$, BGA71$_{NT24}$, BGA67b$_{JHM1114}$, and BGA71b$_{JHM1114}$ blocked formation of C9 polymers indicating that these molecules terminate complement at the final step of the cascade.

To examine the mode of action, serum protection assays with purified proteins were conducted. These analyses revealed that BGA68$_{NT24}$, BGA67b$_{JHM1114}$, and BGA71b$_{JHM1114}$ protect serum-sensitive spirochetes from complement-mediated lysis. Three gain-of-function strains producing BGA66$_{NT24}$, BGA67b$_{JHM1114}$ or BGA71$_{NT24}$ were also generated. All transformants challenged with 50% human serum survived indicating that the complement inhibitory capacity of BGA66 and BGA71 orthologs is highly conserved among *B. bavariensis* isolates. Furthermore, Asian *B. bavariensis* display a higher diversity on orthologs belonging to the PFam54 protein family that terminate complement activation but may have additional function in pathogenesis.

O 029

Host immune status and host sex influence the abundance of *Borrelia burgdorferi* in host tissues and transmission to feeding ticks

C. Koloski[1], *G. Hurry*[1], *A. Foley-Eby*[1], *M. Voordouw*[1]
[1]University of Saskatchewan, Veterinary Microbiology, Saskatoon, Canada

The tick-borne spirochete *Borrelia burgdorferi* has efficient transmission from infected vertebrate reservoir hosts to feeding immature blacklegged ticks (*Ixodes scapularis*). Experimental infection studies have shown that the host-to-tick transmission (HTT) of *B. burgdorferi* often decreases over the course of infection. Correlational studies have shown that HTT depends on spirochete load in the host tissues, but to date no one has demonstrated causation by manipulating the latter. Previous studies have shown that mice with severe combined immunodeficiency (SCID) have higher spirochete loads in their tissues compared to immunocompetent wild-type (WT) mice. The purpose of the present study was to compare whether the difference in host tissue spirochete loads between SCID and WT mice influence HTT and the loads acquired by immature *I. scapularis* ticks. WT and SCID mice with a C57BL/6 genetic background were experimentally infected with *B. burgdorferi* via tick bite and subsequently infested with *I. scapularis* larvae at weeks 4, 8, and 12 post-infection (PI) to measure HTT. Mice were euthanized and organs were dissected at week 16 PI. We used qPCR to determine the infection status and the spirochete loads in the ticks and mouse tissues. The spirochete loads in the tissues of the SCID mice were 260x higher compared to WT mice. The infection prevalence of *B. burgdorferi* in immature *I. scapularis* ticks that fed on SCID mice was 100.0%, whereas it decreased in WT mice over time. The spirochete load in larvae that had fed on SCID mice was 24x higher compared to the larvae that fed on WT mice but declined to a 3-fold difference after the larvae had moulted into nymphs. We also found that male mice had higher tissue spirochete loads compared to female mice for both mouse genotypes. This research will enhance our understanding of how interactions between the host immune system and the spirochete load in the host tissues influence transmission of this pathogen to feeding ticks.

O 030

Borrelia garinii – does genome type reflect host association?

G. Margos[1], *S. Hepner*[1], *M. Hofmann*[1], *M. Dupraz*[2], *C. Hartberger*[1], *A. Sing*[1], *K. McCoy*[2]
S. Casjens[3], *V. Fingerle*[1]
[1]Bavarian Health and Food Safety Authority, German National Reference Centre for Borrelia, Oberschleissheim, Germany
[2]University of Montpellier, MIVEGEC, Montpellier, France
[3]University of Utah, Microbiology and Immunology, Salt Lake City, UT, United States

Borrelia burgdorferi sensu lato is a species complex of spirochetal bacteria that occupy different ecological niches reflected in their reservoir host- and vector-associations. *Borrelia* genomes comprise a linear chromosome and numerous linear and circular plasmids. Proteins encoded by plasmid genes

play a major role in host-vector interactions and potentially in niche adaptation. Plasmid types (defined by PFam32 loci) present in strains may vary even within a single species and may indicate niche adaptation. *Borrelia garinii* is common in Europe (vector *Ixodes ricinus*), Asia (vector *I. persulcatus*) and in marine birds (vector *I. uriae*). For the latter, only a single isolate and its genome were available. Using *B. garinii* from marine bird transmission cycles, we investigated if genome structural diversity is a general pattern in *Borrelia* and if there is a correlation between genome type and host species. *Ixodes uriae* collected from avian hosts and their nests served to initiate cultures. Genomes were assembled from sequences generated by Illumina (N=10) and PacBio (N=1) technologies using SPAdes, CLC, and HGAP. Plasmid types were defined by PFam32 or related loci.

Ten out of the 17 novel isolates were sequenced. Five isolates belonged to the same MLST sequence type (ST575) as the reference Far04 and one (ST804) was a single locus variant (SLV). The others belonged to different clonal complexes suggesting several independent entries into the marine cycle. The 10 genomes segregated into 5 different genome types, being defined by plasmid types. We show that i) these genomes of *B. garinii* contain fewer plasmids (6-9) than *B. garinii* from terrestrial avian species (generally $\geq$10) but that ii) genome type did not match host species association.

In conclusion, we confirm a high genome diversity in *B. garinii*. More work will be required to understand (i) how the plasmid diversity generated and maintained; (ii) how this diversity relates to host- and/or vector-adaptation.

O 031

The role of hosts in shaping highly structured *Borrelia lusitaniae* populations.

A. C. Norte[1], I. Lopes de Carvalho[2], M. A. Carretero[3,4,5], L. P. da Silva[3,4], E. M. Laghzaoui[6]
E. H. El Mouden[6], R. Afonso[7], F. J. Zamora-Camacho[8], J. Garrido-Bautista[9], G. Moreno-Rueda[9]
J. A. Ramos[1], P. Boyer[10], S. Castillo-Ramirez[11], M. Chvostac[12], M. O Brahami[13], R. E. Rollins[14]
T. Woudenberg[15], Y. M. Didyk[12,16], M. Derdáková[12], M. S. Núncio[2], V. Fingerle[15], G. Margos[15]
[1]MARE – Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[2]National Institute of Heatlh Doutor Ricardo Jorge, Centre for Vector and Infectious Diseases Research, Setúbal, Portugal
[3]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Vairão, Portugal
[4]BIOPOLIS Program in Genomics, Biodiversity and Land Planning, Vairão, Portugal
[5]University of Porto, Departamento de Biologia, Faculdade de Ciências, Porto, Portugal
[6]Laboratory of Water, Biodiversity and Climate Change, Faculty of Science Semlalia, Cadi Ayyad University, Marrakech, Portugal
[7]Conservation Biology Research Group, Department of Anatomy, Cell Biology and Zoology, University of Extremadura, Badajoz, Spain
[8]University Complutense de Madrid, Madrid, Spain
[9]University of Granada, Zoology, Granada, Spain
[10]University of Strasbourg, Lyme Borreliosis Group, ITI InnoVec, Fédération de Médecine Translationnelle de Strasbourg, Institut de Bactériologie, Strasbourg, France
[11]Universidad Nacional Autónoma de México, Centro de Ciencias Genómicas, México, Mexico
[12]Slovak Academy of Sciences, Zoology, Bratislava, Slovakia
[13]Laboratory of Ecology and Biology of Terrestrial Ecosystems, Faculty Biological and Agronomic Sciences, University Mouloud Mammeri, Tizi Ouzou, Algeria
[14]LMU Munich, Faculty of Biology, Division of Evolutionary Biology, Munich, Germany
[15]National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany
[16]Department of Acarology, Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, Ukraine

Vertebrate reservoir hosts and tick vector species are supposed to shape the populations of *Borrelia burgdorferi* s.l. species. Reservoir hosts include some common species of small mammals, birds and lizards. The lizard-associated *B. lusitaniae* is mainly distributed around the Mediterranean basin and has focal distributions in northern and central Europe. Our genetic characterization of *B. lusitaniae* infecting ticks across Europe and northern Africa using Multilocus Sequence Typing (MLST) revealed that this species has highly structured populations in Portugal: those from southern Portugal were closely related to those in northern Africa, and populations from northern Portugal were genetically closer to those of central and northern Europe. The population structure of the Portuguese/African clade partially reflected that of its main *Ixodes* vectors, which was determined by genotyping a mitochondrial and a nuclear gene, suggesting some co-evolution. However, vertebrate hosts may also play a role in shaping *Borrelia* populations: the Algerian sand racer *Psammodromus algirus*,

ubiquitous around the Mediterranean basin, was previously proved as a reservoir host for *B. lusitaniae* while other lizard species are competent reservoirs for this bacterial species in central and northern Europe. Interestingly, *P. algirus* is genetically structured into well differentiated lineages across its range. To better understand the impact of this lizard's population structure on *B. lusitaniae* we collected tail tissues and ticks infesting *P. algirus* in northern Africa, southern Spain and both southern and northern Portugal. *Borrelia lusitaniae* infecting these ticks and tissues was characterised by MLST and *P. algirus* lineages and populations were identified through genotyping two mtDNA markers. This simultaneous characterization of host and bacterial populations allows elucidating how vertebrate reservoir hosts may impact the phylogeny and evolution of tick-transmitted pathogens.

O 032

Development and validation of a protein array for Lyme borreliosis diagnostics

O. Stukolova[1], *J. Ursinus*[2], *D. Hoornstra*[2], *L. Karan*[1], *M. Markelov*[3], *A. Dolgova*[1], *A. Sudina*[1]
A. Platonov[1], *J. Hovius*[2]
[1]CRIE, Moscow, Russian Federation
[2]Amsterdam University Medical Centers, Amsterdam, Netherlands
[3]Izmerov Research Institute of Occupational Health, Moscow, Russian Federation

1. Introduction: For Lyme borreliosis (LB) diagnostics two-tier testing is recommended. This is laborious, prone to inter-observer variability, and expensive.
2. Objectives: We aimed to develop and validate a one-tier protein microarray for LB diagnostics.
3. Materials & Methods: Specific IgM and IgG antibodies were detected using a microarray containing 12 *Borrelia afzelii* and *Borrelia bavariensis* (Bbsl) antigens. Serum samples of culture-confirmed LB patients were used for validation, consisting of 58 patients with erythema migrans (EM), and 13 patients with disseminated LB in combination with a skin manifestation (DLD). Endemic and non-endemic healthy controls as well as a potentially cross-reactive cohort, 283 sera samples of 83 patients with PCR-proven *Borrelia miyamotoi* disease (BMD) were used. The cut-off for reactivity for each individual antigen was based on ROC-analyses, and an interpretation algorithm was developed by stepwise logistic regression.
4. Results: IgM-positivity was defined as reactivity against OspC or two of the following proteins; VlsE, p58, p39, p41 or BBK32. IgG-positivity was defined as reactivity against VlsE or two of the following proteins; p100, p58, p41, p39, BBK32 or OspC. A sensitivity of 48% was observed in acute samples of EM patients, and 95% in late samples of disseminated LB for IgM/IgG. Regarding the EM cohort, the overall protein microarray sensitivity was 66% and concerning the disseminated LB cohort 92% for IgM/IgG. Specificity was 96% for IgM/IgG in the healthy controls. Furthermore, the protein microarray distinguished BMD from LB patients, with a specificity of 98% (CI 95% 95-100) for IgM, and 97% for IgG.
5. Conclusion: We have developed and validated a novel protein microarray for LB diagnostics with an appropriate sensitivity and excellent specificity. This novel protein microarray might be a valuable contribution to routine LB diagnostics.

O 033

Lyme borreliosis and health care system use in Germany – a retrospective claims data analysis in 2015–2019

C. Hagemann[1], G. Brestrich[1], J. Diesing[2], N. Kossack[2], D. Häckl[2], A. Pilz[3], J. Stark[4], H. Yu[4], J. Süß[5]
[1]Pfizer Pharma GmbH, Outcomes Research, Berlin, Germany
[2]WIG2 GmbH, Leipzig, Germany
[3]Pfizer Corporation Austria GmbH, Vienna, Austria
[4]Pfizer Inc., Collegeville, PA, United States
[5]Brehm Memorial Center (BREHM WORLD), Renthendorf, Germany

Introduction: Lyme borreliosis (LB) is a tick-borne disease caused by the spirochete *Borrelia burgdorferi sensu lato*. We assessed epidemiology and health care resource utilization (HCRU) in a statutory health-insured (SHI) population with disseminated and non-disseminated LB in Germany.

Methods: Applying a combination of ICD-10 codes, antibiotic prescriptions, and/or laboratory test orders to describe LB and its manifestations, we analyzed health claims data of on average 3.2 million SHI members per year. We then compared health care resource utilization (in- and outpatient visits, medication) over 3 years following an incident LB diagnosis in 2015 and 2016 using 1:4 propensity score matching (age, sex, comorbidities, and region) to compose a control population.

Results: Overall LB incidence was 255/100,000 population in 2016. There were significantly more outpatient visits from LB cases compared to their matched controls in the three years after initial diagnosis (4.55 in year 1; 1.22 in years 2 and 3; $p<0.001$ across all years), and 0.06 ($p<0.001$) additional inpatient admissions per LB patient in the first year. Lyme arthritis (LA) and Lyme neuroborreliosis (LNB) cases had also a significantly longer hospital stay compared to their matched controls in the first year (LA 4.9 vs. 2.6 days, $p<0.001$; LNB 10.0 vs. 3.1 days, $p<0.001$). When extrapolating incidence to the entire SHI population, ~182k overall LB cases occurred in Germany in 2016, amounting to ~1.3 million additional outpatient visits within 3 years after an initial LB diagnosis, and to ~11k hospital admissions in the first year after diagnosis in the SHI population.

Conclusion: LB results in increased utilization of the health care system in both in- and outpatient sectors. Disseminated forms are more frequently treated in hospital, and LNB patients have a particularly longer length of stay. In the future, the expected increase in LB cases due to climate change could further inflate this health care system burden.

O 034

Ixodes ricinus abundance and influencing factors in northern Germany during the years 2021–2022

A. Springer[1], *O. Bozbey*[1], *J. Probst*[1], *A. K. Topp*[1], *I. Bulling*[2], *M. Komorek*[2], *M. Komorek*[2], *O. Kahl*[2] *C. Strube*[1]
[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hannover, Germany
[2]tick-radar GmbH, Berlin, Germany

Introduction: *Ixodes ricinus* is the most important vector of tick-borne pathogens in large parts of Europe. Although there have been numerous field studies on this tick species, how different factors drive its local abundance is not yet fully understood. In northern Germany, only few studies on tick abundance have been conducted so far, as compared to southern parts of the country.
Objectives: We have aimed to assess questing tick abundance across northern Germany and to identify the driving ecological factors in this ongoing study.
Materials and Methods: At 43 sites in 13 northern German areas, questing tick density per 100 m^2 was determined monthly by the flagging method from April to July of 2021 and 2022, i.e. during the main *I. ricinus* activity period. Generalized linear mixed models were used to analyse associations with habitat characteristics and climate data obtained from local weather stations.
Results: Approximately 25,000 *I. ricinus/inopinatus* specimens were collected in total. The average density of questing nymphs ranged from 0.9/100 m^2 to 227.4/100 m^2 depending on the studied site, with a mean number of adult ticks from 0.4 to 29.5/100 m^2. Altogether, higher questing tick densities were observed in 2021 (1.0-359.0 nymphs/100 m^2 on average) compared to 2022 (0.8-149.3 nymphs/100 m^2 on average), but there were differences among different areas and sites. Significant correlations of nymph density with forest type, temperature and precipitation were observed. Moreover, the number of frost days in the preceding winter was significantly associated with nymph density in broadleaved and coniferous forests, but not in mixed and inner-urban forests.
Conclusion: Comparatively high questing tick densities were observed in northern Germany in 2021 and 2022. Monitoring questing tick density will be continued in this so far neglected geographic area in 2023.

O 035

Usefulness of multiomic studies in TBE and bacterial co-infection

A. Moniuszko-Malinowska[1], *E. Skrzydlewska*[1]
[1]Medical University of Bialystok, Department of Infectious Diseases and Neuroinfections, Bialystok, Poland

Question: To describe initial results of multiomic studies on TBE and co-infections (LB and HGA).
Methods: We analysed plasma proteomic and lipidomic profile. In three papers published by us we observed:

1. TBE and co-infections promote plasma ROS enhanced generation and antioxidant defense reduction, especially in relation to glutathione and thioredoxin systems, despite the increased effectiveness of Nrf2 transcription factor in granulocytes. Oxidative stress promotes oxidative modifications of phospholipids containing polyunsaturated fatty acids (LA, AA, EPA) with increased lipid peroxidation (estimated as 8-isoPGF2α, 4-HNE). It is accompanied by protein modifications (4-HNE-protein adducts, carbonyl groups, dityrosine increase, and tryptophan level decrease).
2. Highly elevated groups of proteins in TBE and co-infected patients were involved in the pro-inflammatory response and protein degradation. Antioxidant proteins and factors responsible for protein biosynthesis were downregulated.
3. The levels of phosphatidylcholines and lysophosphatidylcholines were increased. We observed differences in phosphoethanolamines and sphingomyelins between TBE and co-infection.

We have analysed the TAS, lipid peroxidation products, endocannabinoids and eicosanoids in CSF of TBE patients and found that TAS was decreased and accompanied by increased levels of lipid peroxidation products (4-HNE, MDA, isoprostanes and neuroprostanes), major endocannabinoids (AEA and 2AG), and eicosanoids. In co-infections, significant changes in the levels of some lipid mediators were observed even after treatment (data not published yet).

Conclusions: TBE and co-infections promote redox balance disturbances in the plasma and CSF leading to oxidative stress and increased metabolism of phospholipids and proteins. The obtained results allow to distinguish the pathomechanism of TBE from co-infection, and may improve the diagnostic process and enable a more efficient pharmacotherapy.

O 036

Abundance in host tissues drives lifetime transmission to ticks for 11 strains of *Borrelia burgdorferi*

C. Zinck[1], *M. Voordouw*[1]
[1]Western College of Veterinary Medicine, Veterinary Microbiology, Saskatoon, Canada

Life history theory for pathogens assumes a positive relationship between pathogen transmission and pathogen abundance in the host tissues. This study investigated this relationship for a set of 11 field-collected strains of the tick-borne spirochete *Borrelia burgdorferi* using a model rodent host (*Mus musculus,* C3H/HeJ) and the blacklegged tick (*Ixodes scapularis*). To determine the relationship between pathogen abundance and lifetime host-to-tick transmission (HTT), male and female mice were experimentally infected with 1 of 11 strains of *B. burgdorferi*. Lifetime HTT was measured by infesting mice with *I. scapularis* larvae at days 30, 60, and 90 post-infection (PI). Abundance of each strain in host necropsies at 97 days PI was estimated using qPCR. The prevalence and abundance of the strains in immature *I. scapularis* (fed larvae and resultant nymphs) was estimated using qPCR. Among the 11 strains of Bbss, there was significant variation in host tissue spirochete load and in lifetime HTT. Strains with higher host tissue spirochete loads transferred a larger dose of spirochetes to feeding larvae. After moulting into nymphs, these same ticks had a higher probability of being infected and carried higher spirochete loads. Finally, we found that our laboratory-based estimates of lifetime HTT were predictive of the frequencies of these strains in nature (obtained from the literature). For Bbss, the strains that establish high abundance in hosts have high lifetime transmission and are the strains that animal hosts and humans are most likely to encounter in nature.

O 037

Host-pathogen interactions and antimicrobial defenses by innate immune receptor NLRX1 during Lyme borreliosis

J. D. Tupik[1], *M. McClune*[2], *H. W. Camp*[1], *J. A. Gregory*[1], *J. Dressler*[2], *J. W. Markov Madanick*[1]
M. A. Nagai-Singer[1], *B. Jutras*[2], *I. C. Allen*[1,3]
[1]Virginia-Maryland College of Veterinary Medicine, Department of Biomedical Sciences & Pathobiology, Blacksburg, VA, United States
[2]Virginia Tech, Biochemistry, Blacksburg, VA, United States
[3]Virginia Tech Carilion School of Medicine, Department of Basic Science Education, Roanoke, VA, United States

Introduction: Lyme borreliosis, caused by the bacterium *Borrelia burgdorferi* s.l., is an emerging infectious disease of global concern. Roughly 60% of untreated patients will develop inflammation of the joints termed Lyme arthritis, resulting from sustained inflammatory signaling by the innate immune system. Currently, there are limited therapeutics for antibiotic-refractory Lyme arthritis, warranting investigation into how our innate immune system can mitigate this inflammation.
Question: Here, we studied how the anti-inflammatory innate immune receptor NLRX1 regulates host-pathogen interactions in response to *Borrelia burgdorferi* (strain B31-A3). Expressed almost

ubiquitously in mammalian cells, NLRX1 senses conserved genetic motifs of pathogens to regulate innate immune pathways. Because NLRX1 modulates inflammatory signaling, cell death, autophagy, and cell metabolism, we hypothesized that NLRX1 could have unique antimicrobial defenses against Lyme arthritis.

Methods & Results: Through 30-day models of infection in novel *Nlrx1*$^{-/-}$ mice, we found that NLRX1 significantly decreased arthritis severity in wildtype mice when compared with knockouts, modulating bacterial load *in vivo*. We next determined in *Nlrx1*$^{-/-}$ murine macrophages that NLRX1 may control *B. burgdorferi* persistence by promoting Reactive Oxygen Species (ROS) mediated-cell death and autophagy, while decreasing cell proliferation. Finally, by infecting novel NLRX1 overexpression human monocytes, we found that elevated NLRX1 significantly decreased pro-inflammatory NF-κB-mediated cytokine secretion.

Conclusions: These results indicate that NLRX1 plays a protective role in mitigating Lyme arthritis in both murine and human models. Further, this protection may occur through NLRX1's modulation of cell death and attenuation of inflammatory signaling. Therefore, we emphasize the importance of NLRX1 sensing of *B. burgdorferi* and encourage its further investigation for new treatments for Lyme arthritis.

O 038

PacBio (HiFi) data need several assembler for completed *Borrelia* plasmid reconstruction

S. Hepner[1,2], *K. Kuleshov*[3], *A. Tooming-Kunderud*[4], *N. Alig*[1,2], *A. Gofton*[5], *S. Casjens*[6], *R. E. Rollins*[7] *A. Dangel*[2], *E. Mourkas*[8], *S. K. Sheppard*[8], *A. Wieser*[9], *J. Hübner*[10], *A. Sing*[1,2], *V. Fingerle*[1,2], *G. Margos*[1,2]
[1]German National Reference Centre for Borrelia, Oberschleissheim, Germany
[2]Bavarian Health and Food Safety Authority, Oberschleissheim, Germany
[3]Central Research Institute of Epidemiology, Moscow, Russian Federation
[4]University of Oslo, Norwegian Sequencing Centre at Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, Oslo, Norway
[5]CSIRO, Health and Biosecurity, Canberra, Australia
[6]University of Utah, Microbiology and Immunology, Salt Lake City, UT, United States
[7]Institute of Avian Research "Vogelwarte Helgoland", Wilhelmshaven, Germany
[8]University of Oxford, Biology, Oxford, United Kingdom
[9]Max von Pettenkofer Institute, Medical Microbiology and Hospital Epidemiology, Faculty of Medicine, Munich, Germany
[10]Dr. von Hauner Children's Hospital, Munich, Germany

Bacteria of the *Borrelia burgdorferi* sensu lato complex are the causative agents of Lyme borreliosis. Different genospecies vary in their tick-vector/host associations and pathogenicity. The genetic basis for these adaptations is unresolved and requires complete genomes for comparative analyses. The *Borrelia* genome consists of a chromosome and linear and circular plasmids, with plasmid genes being involved in host/vector interactions. Assembling complete plasmids is challenging due to the high levels of complexity and homology between plasmid types.

The recent development of high-fidelity (HiFi) PacBio sequencing, which promises >99.9% base calling accuracy, is a significant advancement in long-read sequencing technologies. Using *B. bavariensis*, *B. garinii* and *B. valaisiana*, we investigated if this leads to superior genome assemblies.

We generated PacBio traditional and HiFi data for 27 isolates and used three assemblers for genome reconstruction: 1) microbial, 2) IPA, 3) HiCanu. Several quality control and refinement steps were conducted, and assemblies were compared regarding genome completeness.

The IPA assembler resulted in the lowest contig number but many incomplete plasmids. HiCanu assemblies showed the highest contig numbers (due to duplicates) but resulted in complete plasmids. The microbial assembler performed well as HiCanu. None of the strategies unequivocally performed best for the entire dataset and not even for all elements of a single genome. Plasmids that were incomplete or not recovered from one assembler were sometimes completely reconstructed using another assembler.

Despite the latest available sequencing and assembly technologies, *Borrelia* plasmid reconstruction is still highly complex. Although PacBio HiFi data are promising, careful contig refining steps and comparison to other assembly results are needed. Only by refining, comparing and combining several assembly results for each isolate, we were able to reconstruct complete genomes.

O 039

Detecting RNA viruses in tick cell cultures by SISPA and nanopore sequencing

J. J. Khoo[1], *A. Palomar*[2], *L. Bell-Sakyi*[1]
[1]University of Liverpool, The Tick Cell Biobank, Department of Infection Biology and Microbiomes, Institute of Infection, Veterinary and Ecological Sciences, Liverpool, United Kingdom
[2]Hospital Universitario San Pedro-CIBIR, Centre of Rickettsiosis and Arthropod-Borne Diseases, Logroño, La Rioja, Spain

Introduction: Tick cell lines are known to harbour viruses that could have originated from the parent ticks or from subsequent cross-contamination. For example, St. Croix River virus (SCRV) and *Ixodes scapularis* iflavirus (ISIV) were found in *I. scapularis*-derived cell lines. These viruses may interact with cell biology and modulate co-infections with other microorganisms. Sequence-independent single-primer-amplification (SISPA) and nanopore sequencing have been used to detect pathogenic viruses in ticks and infected mammalian cell cultures. SISPA+nanopore sequencing could potentially provide an effective method for screening for endogenous RNA viruses in tick cell lines.

Objective: To evaluate the utility of SISPA+nanopore sequencing in detecting virus sequences in *Ixodes* spp. cell lines.

Materials and Methods: SISPA+nanopore sequencing was performed on RNA extracts from *I. scapularis* (IDE8, ISE6) and *I. ricinus* (IRE11) cells known to be infected with SCRV and/or ISIV. Metagenomic analysis tools (e.g. Epi2me WIMP, Bugseq, Kraken2) were evaluated for the detection of reads associated with SCRV, ISIV and other RNA virus families. Assembly of the virus sequences was attempted. BLAST analyses were performed to determine the identities of the assembled contigs.

Results: All metagenomic analysis tools assigned <5% reads to virus origin. *Orthornavirae* (encompassing SCRV and ISIV) sequences were detected in all cultures. However, only SCRV was detected at “species” level using these tools. The presence of ISIV sequences could only be determined using

BLAST analyses, which also detected the presence of bunyavirus sequences.
Conclusions: Virus sequences were recovered from the SISPA+nanopore sequencing data for the tick cell lines tested; however, the protocol needs to be further optimised especially for mass testing of cell lines with unknown infection status. The advantages, limitations and possible improvements to the experimental protocol will be discussed.

O 040

The roles of vitellogenins in *Babesia*-infected *Haemaphysalis longicornis* ticks

R. Umemiya-Shirafuji[1], *N. Sato*[1], *M. Kuniyori*[1], *H. Suzuki*[1]
[1]Obihiro University of Agriculture and Veterinary Medicine, National Research Center for Protozoan Diseases, Obihiro, Japan

Question: The molecular mechanisms underlying *Babesia* infection in tick oocytes remain unknown. We hypothesized that there are interactions between oogenesis-related molecules and *Babesia* molecules during the preovipositional period when active oogenesis progresses in female ticks.
Methods: To find the key molecule(s) for *Babesia* infection in ticks, we focused on the yolk protein precursor (vitellogenin; Vg) and its related molecules. Firstly, after the semiartificial mouse skin membrane feeding of *Babesia ovata*-infected bovine red blood cells, the expression profiles of *Vg* and its related genes of *Haemaphysalis longicornis* were analyzed by real-time PCR using tissues collected during the preovipositional period. Subsequently, *Vg* knockdown was performed and *Babesia* DNA in the ovary and hemolymph was detected by nested PCR.
Results: The expression levels of *Vg-2* and *Vg-3* decreased in the fat body of *Babesia*-infected *H. longicornis* 1 day after engorgement (DAE). In the ovary, *Vg-2* expression was significantly higher in *Babesia*-infected ticks than in uninfected ticks 1 and 2 DAE and decreased at 3 DAE. Compared with uninfected ticks, *Vg-1* expression in the midgut significantly increased in *Babesia*-infected ticks 1 and 2 DAE. *Vg* receptor gene expression was significantly lower in *Babesia*-infected ticks than in uninfected ticks 2 and 4 DAE. Autophagy-related gene 6 had a lower gene expression level in *Babesia*-infected ticks compared to uninfected ticks at 2 DAE. Western blotting revealed *Vg*-2 accumulation in the fat body and hemolymph of *Babesia*-infected ticks. Moreover, *Vg-2* knockdown ticks had a lower detection rate of *B. ovata* DNA in the ovary and a significant reduction of *B. ovata* DNA in the hemolymph compared with control ticks.
Conclusions: Our results suggest that accumulated *Vg*-2 is related to *Babesia* infection in the tick. *Vg-2* might be involved not only in oogenesis but also in the survival of *Babesia* in the hemolymph and/or infection in tick oocytes.

O 041

Rickettsial determinants for tick infection and transmission

K. Macaluso[1]*, N. Tongluan*[1]*, C. Suwanbongkot*[1]
[1]University of South Alabama College of Medicine, Microbiology and Immunology, Mobile, AL, United States

Question: Studies have implicated several rickettsial determinants, including surface cell antigen-0 (Sca0), Sca1, Sca2, Sca4, Sca5, and RickA, in adhesion, invasion, cell-to-cell spread, and/or avoidance of the immune response in a mammalian host system. Much less is known about the role of rickettsial proteins in the tick vector. It is hypothesized that if a rickettsial protein is essential to tick infection and subsequent transmission, then disruption of the functional protein will generate an altered infection phenotype. Using rickettsial mutants, growth characteristics in vitro and in vivo seek to identify rickettsial molecules required for tick infection.
Methods: *Rickettsia parkeri* wild-type (strain Portsmouth) or one of the characterized *R. parkeri sca*::tn (non-functional Sca) mutants were infected in tick-derived ISE6 for *in vitro* growth kinetics analysis. *In vivo*, tick infection and transmission bioassays were performed by capillary feeding rickettsiae into the naïve *Amblyomma maculatum* ticks, the natural host for *R. parkeri*. Comparisons between wild-type and mutants were assessed by microscopy and qPCR.
Results: Growth kinetics in tick cell lines varied depending on the mutation. Rickettsiae lacking function OmpB had a similar phenotype as the wild-type bacteria. However, a mutation in Sca4 resulted in a slower growth phenotype compared to wild-type. In the tick vector, mutations consistently resulted in decreased rickettsial loads when compared to wild-type.
Conclusion: The data indicate that while not essential for vector infection, rickettsial molecules contribute to the infection and dissemination process. The distinct infection and dissemination profiles illuminate the temporal and tissue-specific role of rickettsial proteins in tick infection.

O 042

Avian *Babesia* – a description of *Babesia* sp. YLG from yellow-legged gulls and its transmission by *Ornithodoros maritimus*

C. Bonsergent[1]*, M. Vittecoq*[2]*, C. Leray*[2]*, M. Jouglin*[1]*, K. McCoy*[3]*, L. Malandrin*[1]
[1]INRAE, UMR BIOEPAR, Nantes, France
[2]Tour du Valat, Research Institute for the Conservation of Mediterranean Wetlands, Arles, France
[3]University of Montpellier, MIVEGEC, Montpellier, France

Introduction: The few avian *Babesia* described to date cluster into two distinct lineages, the Kiwiensis (*Babesia* sensu stricto clade) and the Peircei group (clade V). The role of ticks in the transmission of these species is largely unknown.

Objectives: Our objective is to increase knowledge on the geographical distribution, host range and biological features of avian *Babesia*. Here, we report data from a piroplasm infecting yellow-legged gulls in France, and its transmission by the soft tick *Ornithodoros maritimus*.

Materials and methods: Blood samples from yellow-legged gull chicks and *O. maritimus* ticks were collected from the Carteau islet located in the Camargue area of southern France. Piroplasms were morphologically described from blood smears. Molecular characterization was performed using the 18S rRNA and *cox1* genes.

Different instars of *O. maritimus,* collected from nests, were dissected. Detection and molecular characterization of piroplasm DNA was then performed on separate organs to study the parasite transmission route.

Results: Intraerythrocytic pale, large and pleomorphic forms were observed on blood smears with a prevalence in chicks of 80% (16/20) in 2019 and 58% (21/36) in 2020. The 18S rRNA sequencing resulted in 16 identical sequences (1461-1485 pb) and phylogenetic analysis placed this new *Babesia,* named *Babesia* sp. YLG, in the Peircei group.

The analysis of 144 *O. maritimus* ticks collected between 2019 and 2022 revealed a prevalence of *Babesia* sp. YLG of 27.8%. In terms of potential transmission routes, Babesia sp. YLG DNA was detected in 12.4% of the tested salivary glands (17/137), 9.5% of the ovaries (10/105), 9.1% of the male genitalia (1/11) and 9.1% of the endospermatophores (6/66).

Conclusion: We report the description of a new *Babesia* species from the Peircei group infecting a large gull in Europe. We demonstrate its transmission to chicks via the soft tick *O. maritimus* and raise the possibility of transovarial as well as sexual transmission.

O 043

A rapid and economical tool for the comprehensive identification of ticks, pathogens, and hosts, and their infection dynamics

G. Chauhan[1,2], O. Aydemir[1], R. DeFeo[3], D. Genereux[2], J. Bailey[3], R. Daniels[1,2,4], E. Karlsson[1,2,5]
[1]UMASS Chan Medical School, Bioinformatics and Integrative Biology, Worcester, MA, United States
[2]Broad Institute of MIT and Harvard, Vertebrate Genomics, Cambridge, MA, United States
[3]Brown University, Department of Pathology and Laboratory Medicine, Providence, RI, United States
[4]Harvard T.H. Chan School of Public Health, Department of Immunology and Infectious Diseases, Boston, MA, United States
[5]UMASS Chan Medical School, Program in Molecular Medicine, Worcester, MA, United States

Globally, ticks transmit more pathogens than other arthropods. Tick-borne pathogens include around 60 bacteria, 30 parasites and 100 viruses, one-third of which cause zoonoses. Current methods for tick and pathogen detection are generally time-consuming, error-prone and do not detect co-infections and novel tick-pathogen associations. Moreover, economical methods are lacking for the detection of pathogens in individual ticks. Thus, new epidemiologic investigative tools are needed to simultaneously identify tick species and tick-borne pathogens. Thus, we developed a high-throughput, targeted sequencing-based method using molecular inversion probes (MIPs) to identify known tick-borne pathogens worldwide. We designed 215 host (tick and mammalian) and 320 pathogen MIPs to identify >100 *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus* tick species, >100 tick-borne prokaryotic (*Borrelia*, *Borreliella*, *Ehrlichia*, *Anaplasma*, *Rickettsia*, *Francisella*, *Bartonella* and *Coxiella*) and eukaryotic (*Babesia* and *Theileria*) pathogens and 3 viruses. We also designed MIPs to detect 5 variants associated with *Babesia microti* relapse and identify mammalian hosts to assess tick feeding patterns. These MIPS showed high sensitivity (as low as 1000 copies per reaction) and selectivity. Evaluation in 568 individual adult ticks collected by citizen scientists from across the USA successfully identified the species of 441 ticks. We also identified pathogens in 46 individual ticks; these included B. *burgdorferi* s.l., B. miyamotoi, *A. phagocytophilum*, *E. chaffeensis*, *F. tularensis*, nine different pathogenic species of *Rickettsia*, and the parasite species *B. duncani*. We also detected unclassified *Rickettsia* species in 146 tick samples. This rapid and economical tool allows the comprehensive testing of tick-borne pathogens and can be customized to accommodate new or emerging pathogens for surveillance and epidemiological studies and inform clinical decisions.

O 044

Transcriptomic analysis of *Amblyomma hebraeum* ticks infected with *Ehrlichia ruminantium*

D. Omondi[1], *E. Zweygarth*[1], *E. Murungi*[2], *A. Nijhof*[1]
[1]Freie Universität Berlin, Berlin, Germany
[2]Kisii University Kenya, Kisii, Kenya

The south African bont tick *Amblyomma hebraeum* is a hematophagous vector for the heart-water disease pathogen *Ehrlichia ruminantium* in southern Africa. During feeding, the tick's enterocytes express proteins that perform key functions in blood digestion, including proteins that may be involved in *E. ruminantium* acquisition, colonization and transmission. To delineate the molecular mechanism of midgut response to *E. ruminantium* acquisition and advance new strategies for tick and heartwater control, we performed comparative analyses of midgut transcriptomes of *E. ruminantium* infected engorged *A. hebraeum* nymphs, and infected adult male and female ticks with their corresponding matched uninfected controls, prior to and during feeding. Unigenes were annotated in public databases and their expression levels analyzed for unfed and partially fed adult ticks. A total of 23,632 differentially expressed genes (DEGs) in midguts of engorged nymphs, unfed and partially fed males and females were identified, of which 14,343 were up-regulated and 9,289 were down-regulated in the infected ticks. Annotation of differentially expressed genes revealed their involvement in vital diverse metabolic and cellular processes including digestion of blood proteins, carbohydrates and lipids. Notably, there was up-regulation in expression of genes in the midgut involved in tick immunity, oxidative stress responses and glycoproteins that *E. ruminantium* could potentially use as docking sites for host cell entry. Insights uncovered in this study offer a platform for further investigations into the molecular interaction of *E. ruminantium* and *A. hebraeum*.

O 045

Tick-borne pathogens and body condition of cattle in smallholder rural livestock production systems in East and West Africa

M. Madder[1], *J. Fourie*[2], *D. Heylen*[3]
[1]Clinglobal, Tamarin, Mauritius
[2]Clinvet International Pty (Ltd), Waverly, IA, United States
[3]Institute of Tropical Medicine, Department of Biomedical Sciences, Antwerp, Belgium

Introduction: Most of the African population lives in rural areas and depend on crop and livestock production for their livelihoods.
Objectives: Given their socio-economic importance, we initiated a standardized multi-country surveillance study to assess the current status of tick-borne haemoparasites of cattle in sub-Saharan Africa.
Materials and methods: We assessed pathogen prevalences in the blood of 6,447 animals. In addition, we screened for intrinsic (sex, weight, body condition) and extrinsic (husbandry, tick exposure) risk factors as predictors of infections with TBHPs.
Results: There was a large macro-geographic variation observed in *A. marginale, B. bigemina, B. bovis, E. ruminantium* prevalences. Most correlated with the co-occurrence of their specific sets of vector-competent ticks. Highest numbers of infected cattle were found in Ghana and Benin, and lowest in Burkina Faso. While *T. parva* was seldomly found (Uganda only: 3.0 %), *A. marginale* was found in each country with a prevalence of at least 40 %. *Babesia bovis*-infected individuals had lower body condition scores. Age (as estimated via body weight) was higher in *A. marginale*-infected cattle, but was negatively correlated with *B. bigemina* and *E. ruminantium* prevalences. *Ehrlichia ruminantium* infection was more often found in males, and *A. marginale* more often in transhumance farming. High levels of co-infection, especially the combination *A. marginale* × *B. bigemina*, were observed in all countries, except for Uganda and Burkina Faso.
Conclusions: Tick-borne pathogens of cattle are ubiquitous in African's smallholder cattle production systems. Our standardized study will help a wide range of stakeholders to provide recommendations for TBHP surveillance and prevention in cattle, especially for *B. bovis* which heavily impacts production and continues its spread over the African continent via the invasive *Rhipicephalus microplus* tick.

O 046

Secreted *Theileria annulata* effector protein Ta9 binds and potentially activates proto-oncogenic macrophage Hck.

S. Tajeri[1], *J. Kinnaird*[2], *K. Elati*[1], *B. Shiels*[2], *G. Langsley*[3], *A. Nijhof*[1]
[1]Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany
[2]University of Glasgow, School of Biodiversity, One Health and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, Glasgow, United Kingdom
[3]Institute Cochin, 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. We have previously shown that 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. Since Hck is reported to be a lysosomal marker for a distinct subpopulation of lysosomes in macrophages we tested if Ta9 co-localized with other lysosomal markers LAMP2 and LAMP3. However and interestingly, Ta9+ vesicles were both LAMP2 and LAMP3 negative. Overexpression of full-length GFP-tagged Ta9 in mouse 3T3 cells results in the appearance of numerous spike-like membrane protrusions ("hairy phenotype") and dorsal ruffles. These results are consistent with Ta9 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.

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

O 047

Effects of feeding time on occurrence and quantity of *Borrelia* species and *Babesia* species in ticks detached from roe deers

P. Wilhelmsson[1,2]*, J. Bergqvist*[1]*, P. Kjellander*[3]*, H. Fredlund*[4]*, M. Christensson*[3]*, P. E. Lindgren*[1]
[1]Linköping University, Division of Inflammation and Infection, Department of Biomedical and Clinical Sciences, Linköping, Sweden
[2]Linköping University, Department of Clinical Microbiology, Jönköping, Region Jönköping County, and Department of Biomedical and Clinical Sciences, Linköping, Sweden
[3] Swedish University of Agricultural Sciences, Grimö Wildlife Research Station, Department of Ecology, Riddarhyttan, Sweden
[4]Örebro University, Department of Laboratory Medicine, Faculty of Medicine and Health, Örebro, Sweden
**These authors contributed equally to this work.*

The importance of the European roe deer (*Capreolus capreolus*) for the maintenance of the most prevalent tick species in Northern Europe, *Ixodes ricinus*, suggests that itfurthermore plays a role in transmission of tick-borne pathogens. This study aimed at shedding light on the potential role of the roe deer in transmission of *Borrelia* spp. and *Babesia* spp. Totally, 576 ticks, mainly nymphs, detached from ears of 27 roe deer from the Örebro County, Sweden, were collected. Tick feeding duration of each nymph and adult female tick was estimated based on the scutal and coxal indices. The ticks were analysed using real-time PCR to detect and quantify these pathogens followed by species identification by sequencing PCR-products. 16 % of the ticks contained *Borrelia* spp. and 5.9 % contained *Babesia* spp. We found that the prevalence of *Borrelia* spp. in the ticks decreased significantly with their feeding time. No significant change was observed in the number of *Borrelia* spp. cells with feeding time. The prevalence of *Babesia* spp. remained steady with no significant difference in parasite gene copies with feeding time. This study indicates that the roe deer may play a role in the transmission, by negatively affecting the survival of *Borrelia* spp. in I. ricinus ticks, whilst no such effect was detected for *Babesia* spp.

O 048

Tick systematics – current trends and future directions

B. Mans[1]
[1]Agricultural Research Council, Onderstepoort Veterinary Research, Epidemiology, Parasites and Vectors, Onderstepoort, Pretoria, South Africa

Prior to the twenty-first century, tick taxonomy and systematics were largely directed by biological and morphological expertise. The advent of molecular approaches in the 1990s proved to be game changing for tick systematics with many classifications being overthrown or modified. As more molecular data are generated this trend continues with constant surprising findings, raising the question on where molecular systematics is heading and what this may hold for the future of tick taxonomy and classification. The question is also raised on how classic and modern taxonomy and systematics may be reconciled. A major outcome necessary for an understanding of tick evolution is a robust supported stable taxonomical framework that would allow interpretation of lineage specific characters within its systematic context. The current study unpack these issues and provide perspectives on how current and future advances in knowledge and technology may impact our past and future understanding of tick evolution and systematics.

O 049

What have we learned from the first 630 mitochondrial genomes of ticks and other Acari?

S. Barker[1], *S. Kelava*[1]
[1]University of Queensland, Parasitology, St Lucia, Australia

Mitochondrial genomes have been remarkably instructive about the evolutionary-history (phylogeny), population-genetics and phylogeography of Acari, particularly the ticks. At present we have entire mt genomes for 145 of the 896+ species of ticks (336 mt genomes in total), and for 146 of the thousands of other species of Acari (296 mt genomes in total). Total number of mt genomes available for the Acari is 632. It has never been easier to sequence entire mt genomes. Any lab with basic wet-lab capability can do this by using commercial sequencing companies. In 2021, Barker & Kelava precipitated the Tick Mitochondrial Genome Network with a YouTube Channel of the monthly meetings [https://www.youtube.com/channel/UCnBhfhYxjC4rsJmVpBwHT0g/featured]. This is a worthwhile resource for people wanting to join our enterprise.
Selected insights and outcomes will be discussed in our talk, including: (i) phylogenetic trees that led Ben Mans and us to propose that the genus *Carios* s.l. be dissolved and the subgenera *Alectorobius*, *Antricola*, *Nothoaspis*, *Reticulinasus* and *Subparamatus* be raised to genus level; (ii) an extraordinarily re-arranged mt genome arrangement in *Amblyomma* (*Africaniella*) *transversale* that, among other evidence, prompted the elevation of the subgenus *Africaniella* back to a genus; (iii) recent insights into the phylogeny of the genera *Robertsicus*, *Amblyomma* and *Haemaphysalis*; (iv) the tick-box motif may be involved in the insertions of 132 to 312-bp in two *Haemaphysalis* species (*H.* (*Al.*) *inermis* and *H.* (*Al.*) *kitaokai*) and *R.* (*B.*) *geigyi*; (v) assignment of several ticks to subgenera including: *Ixodes woylie* and *I. barkeri* to *Endopalpiger*; (vi) realization that *Ixodes anatis*, the kiwi tick, may be closely related to the ticks of marsupials of Australia and Papua New Guinea.

O 050

Seventy-eight entire mitochondrial genomes and nuclear rRNA genes provide insight into the phylogeny of the hard ticks, particularly the *Haemaphysalis* species, *Africaniella transversale* and *Robertsicus elaphensis*

S. Kelava[1], *S. Barker*[1]
[1]University of Queensland, School of Chemistry and Molecular Biology, Brisbane, Australia

Introduction: Hoogstraal and Kim (1985) proposed from morphology, three groups of *Haemaphysalis* subgenera: (i) the "structurally advanced"; (ii) the "structurally intermediate"; and (iii) the "structurally primitive" subgenera. The phylogeny of *Haemaphysalis*, however, has not yet been resolved.
Objective: We aimed to resolve the phylogeny of the genus *Haemaphysalis*, with respect to the subgenus *Alloceraea*.
Materials & methods: We present 15 mitochondrial genomes and four 18S and 28S rRNAs sequences which were sequenced by long and short-range PCR and Illumina. We constructed phylogenies from 10 mitochondrial protein-coding genes, cox1, and 18S and 28S nuclear rRNA.
Results: We found a 132-bp insertion between tRNA-Glu (E) gene and the *nad1* gene in the mt genome of *Haemaphysalis* (*Alloceraea*) *inermis*. The subgenus *Alloceraea* was in a clade that was separate from the rest of the *Haemaphysalis* ticks. Either *Africaniella transversale* or *Robertsicus elaphensis*, or perhaps *Af*. *transversale* plus *Ro*. *elaphensis*, appear to be the sister-group to the rest of the metastriate Ixodida.
Conclusion: Our *cox1* phylogenies did not indicate monophyly of the "structurally primitive", "structurally intermediate" nor the "structurally advanced" groups of *Haemaphysalis* subgenera. *Alloceraea* may be the only monophyletic subgenus of the genus *Haemaphysalis* sequenced thus far. All of our mt genome and cox1 phylogenies had the subgenus *Alloceraea* in a clade that was separate from the rest of the *Haemaphysalis* ticks. *Alectorobius capensis* from Japan had a higher genetic-identity to *A*. *sawaii* than to the *A. capensis* from South Africa. This indicates that *A. capensis* from Japan may be a cryptic species with respect to the *A. capensis* from South Africa.

O 051

Borrelia down under – an update on Australia"s unique *Borrelia* diversity and implications for our understanding of *Borrelia* evolution

A. Gofton[1]
[1]CSIRO, Health and Biosecurity, Canberra, Australia

Borrelia are one of the most significant and well-studied group of tick-borne microorganisms. While in other parts of the world *Borrelia* have intensively been studied since the mid-20th century, in Australia *Borrelia* research is in its infancy. In the late 1950s several studies identified *Borrelia*-like organisms in native Australian wildlife, however, little further work was conducted, and it would be over fifty years until *Borrelia* were confirmed to be present in Australia, with the discovery of *B. tachyglossi*

in echidnas in 2016. This spurred a flurry of research which has resulted in five additional described *Borrelia* species. Nevertheless, our understanding of Australian *Borrelia* is underdeveloped: we are yet to isolate any Australian *Borrelia*, genome sequences are available for only several species, and we lack a proper understanding of their geographic, host, and vector ranges, and their clinical relevance. Australia has a unique biogeographic history and ancient lineages of mammals and reptiles, and ticks. Unsurprisingly, our *Borrelia* fauna is equally as unique, and from the modest data we have, it is clear that Australian *Borrelia* occupy an important position in the evolutionary history of *Borrelia* as basal members of the reptile-associated clade and relapsing fever clades associated with metastriate vectors. This emerging body of work has the potential to re-write our understanding of *Borrelia* evolution and suggests that ancestral *Borrelia* were associated with only the Ixodidae prior to the metastriata-prostriata split, and adoption by Argasidae vectors occurred much later through a vector-switching event from metastriate hosts.
This talk will provide an overview on the work conducted on Australian *Borrelia* to date, including discussing gaps in our knowledge and opportunities for new research, and explore how Australian *Borrelia* may enhance our understanding of *Borrelia* evolution at a global scale.

O 052

Phylogenetic relationships of the *Amblyomma cajennense* complex (Acari: Ixodidae) at mitogenomic resolution

A. P. Cotes Perdomo[1,2], *S. Nava*[3], *L. Castro*[4], *F. Rivera-Paez*[5], *J. Cortes-Vecino*[6], *J. Uribe*[2]
[1]University of South-Eastern Norway, Department of Natural Sciences and Environmental Health, Bo i Telemark, Norway
[2]Museo Nacional de Ciencias Naturales, Biodiversity and Evolutionary Biology Department, Madrid, Spain
[3]Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Santa Fe, Argentina
[4]Universidad del Magdalena, Grupo de investigación Evolución, Sistemática y Ecología Molecular (GIESEMOL), Facultad de Ciencias Básicas, Santa Marta, Colombia
[5]Universidad de Caldas, Grupo de Investigación en Genética, Biodiversidad y Manejo de Ecosistemas (GEBIOME), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Manizales, Colombia
[6]Universidad Nacional de Colombia, Grupo de Investigación Parasitología Veterinaria, Facultad de Medicina Veterinaria y de Zootecnia, Bogota, Colombia

Introduction: The genus *Amblyomma* is the third most species-diverse within the Ixodidae, which has practically half of the species distributed in the Americas, but species of *Amblyomma* are also distributed in Sub-Saharan Africa, Asia, and Oceania. Within the genus, there are several species complexes with veterinary and public health importance. In America, the *Amblyomma cajennense* complex comprises six species with a wide distribution from Texas to northern Argentina and has a great sanitary impact.

Methods: We combined two sequencing techniques to generate complete mitogenomes of species belonging to the *A. cajennense* complex: genome skimming and long-range PCRs sequencing methods.
Results: Seven new mitochondrial genomes were generated for all species of the *A. cajennense* complex, except for *A. interandinum*. Genetic distances between the mitogenomes corroborate the clear differentiation between the five species of the *A. cajennense* complex. The phylogenetic relationships of this species had previously been evaluated by combining partial nuclear and mitochondrial genes and here these relationships are corroborated with a more robust framework of data, which demonstrates that the conjunction of mitochondrial and nuclear partial genes can resolve close relationships when entire genes or genomes are unavailable.
Conclusions: The gene order, structure, composition, and length are stable across these mitogenomes, and they share the general characteristics of Metastriata. The phylogenetic hypothesis for the *A. cajennense* complex reached previously with partial genes (both mitochondrial and nuclear) is corroborated by a mitogenomic dataset and high statistical support. In contrast, the relationships of other *Amblyomma* members are still unstable. Future studies should increase the number of available mitogenomes for this genus, especially for those species from the Indo-Pacific region and Africa, by means of a better understanding of their relationships and evolution process.

O 053

Nuttalliella in Burmese amber – Implications for tick evolution

L. Chitimia-Dobler[1,2]*, J. A. Dunlop*[3]*, S. Handschuh*[4]*, R. Pienaar*[5]*, B. Mans*[5,6,2]
[1]Bundeswehr Institute of Microbiology, Munich, Germany
[2]University of South Africa, Department of Life and Consumer Sciences, Pretoria, South Africa
[3]Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany
[4]University of Veterinary Medicine Vienna, VetCore Facility for Research / Imaging Unit, Vienna, Austria
[5]Agricultural Research Council, Onderstepoort Veterinary Research, Epidemiology, Parasites and Vectors, Pretoria, South Africa
[6]The Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa, Pretoria, South Africa

Ticks are composed of three extant families (Argasidae, Ixodidae and Nuttalliellidae) and two extinct families (Deinocrotonidae and Khimairidae). The Nuttalliellidae possess one extant species (*Nuttalliella namaqua*) limited to the Afrotropics. A basal relationship to the hard and soft tick families and its limited distribution has suggested an origin for ticks in the Afrotropics. The related family Deinocrotonidae has been found in Burmese amber from Myanmar as well as Spanish amber, suggesting a wider distribution of the lineage composed of Deinocrotonidae and Nuttalliellidae. The current study, describe two fossils (male and female) from the family Nuttalliellidae from mid-Cretaceous (ca. 100 Ma) Burmese amber of Myanmar. Since both specimens derive from the same amber deposit, we provisionally assign both to the same species. Affinities of Burmese tick fossils to the Australasian region, specifically related to rifting of the Burma terrane from northern Australia ∼150 million years ago, suggest that *Nuttalliella* had a much wider distribution than its current limited distribution in southern Africa. The distribution of *Nuttalliella* likely stretched from Africa over Antarctica and much of Australia, suggesting that extant members of this family may still be found in Australia. Considerations for the geographic origins of ticks is discussed, with the conclusion that an Afrotropic origin can as yet not be discarded.

O 054

Reemergence of Brazilian spotted fever – the role of human-modified landscapes

M. B. Labruna[1]
[1]University of São Paulo, Faculty of Veterinary Medicine, São Paulo, Brazil

Brazilian spotted fever (BSF), caused by the bacterium *Rickettsia rickettsii*, is an acute and severe tick-borne disease (≈50% fatality rate) affecting humans in southeastern Brazil, where *Amblyomma sculptum* and *Amblyomma aureolatum* act as vectors. Although BSF was first reported during the 1930s, it has greatly reemerged during the last 40 years. Among the *A. sculptum*-associated BSF-endemic areas, capybaras (*Hydrochoerus hydrochaeris*) act as main hosts for all parasitic stages of the tick vector. Because transovarial transmission and filial infection rates of *R. rickettsii* in *A. sculptum* are low, tick infection rates in nature are $<1\%$. Thus, the establishment of *R. rickettsii* in an *A. sculptum* population depends on the overgrowth status of this population, and on the constant supply of amplifying vertebrate hosts (susceptible capybaras), which once primarily infected will develop bacteremia for a few days, at which point they will create new cohorts of infected ticks. Since capybaras develop an immune response that prevents a second bacteremia, the maintenance of an active focus of *R. rickettsii*-*A. sculptum* depends on a high reproduction rate of capybaras and the large size of the *A. sculptum* population. In the *A. aureolatum*-associated BSF-endemic areas, domestic dogs act as main hosts for adult ticks, whereas passerines serve as hosts for immature ticks. In this case, transovarial transmission and filial infection rates of *R. rickettsii* in *A. aureolatum* are high, but the rickettsial infection might be lethal for ticks, resulting in tick infection rates around 10% in nature. Thus, the establishment of *R. rickettsii* in an *A. aureolatum* population is more stable overtime, depending on the supply of amplifying vertebrate hosts (susceptible dogs) only in a medium to long term scenario. The above conditions for maintenance of *R. rickettsii* in *Amblyomma* vectors has been linked to several anthropic changes in the landscape of southeastern Brazil.

O 055

Phenotypic and genotypic characterization of acaricide-resistant *Rhipicephalus Microplus* field isolates from Brazil

G. M. Klafke[1,2], *F. A. Barbieri*[1], *S. D. Canto*[1], *I. B. Rico*[1], *J. Reck*[1,2], *C. P. Tavares*[3], *A. Evans*[4]
L. M. Costa-Junior[3,2]
[1]Instituto de Pesquisas Veterinárias Desidério Finamor – IPVDF, Eldorado do Sul, RS, Brazil
[2]Comissão Especial de Resistência a Antiparasitários – Colégio Brasileiro de Parasitologia Veterinária, Jaboticabal, SP, Brazil
[3]Universidade Federal do Maranhão – UFMA, São Luís, Brazil
[4]Clinglobal, Tamarin, Mauritius

Multiple resistance to acaricides (MRA) is the greatest obstacle to efficient control of the cattle tick, *Rhipicephalus microplus*. MRA has become frequently detected in Brazil, which seriously threatens the cattle industry. Infestations by *R. microplus* are generally abundant and the use of chemical acaricides is intensified, aggravating the problem of resistance. Knowing the background of resistance is essential to design local programs for tick control and public policies aimed at improving the health status of herds and animal welfare. The laboratory detection of resistance is usually carried out with the larval packet test. Although practical, simple and not expensive, this method is time-consuming and take to six weeks to provide results. Molecular assays are not substitute of the bioassays, but can rapidly provide important data regarding the presence of resistant alleles in a population of ticks. The objective of this ongoing study is to generate data about the acaricide resistance phenotypic and genotypic status of *R. microplus* in Brazil. Larval packet tests were carried out in two laboratories to detect resistance against deltamethrin, chlorfenvinphos, amitraz, fipronil and doramectin. Forty-six isolates from six different states were analyzed. The Porto Alegre strain was used as susceptible reference. All the isolates tested were resistant to at least one acaricide. Thirty-three isolates (72%) were resistant to three or more acaricides. The highest frequency of resistance found was to deltamethrin (98%) followed by chlorfenvinphos (86%), doramectin (70%) amitraz (55%) and fipronil (39%). Next step on the study is to investigate the frequency of resistant alleles in the sampled isolates. To the best of our knowledge, this is the first country-wide investigation of phenotypic and genotypic acaricide resistance status for cattle ticks in Brazil and shall provide valuable information for the cattle industry stakeholders regarding rational parasite control.

O 056

Metarhizium robertsii ROS tolerant is effective against *Rhipicephalus microplus* on semi-field conditions combined with an anti-tick vaccine

J. Fiorotti[1], *L. Nobrega Meirelles*[2], *M. Scavassini Peña*[1], *V. Silvestre Borio*[2], *T. E. Galdino Andrade*[1]
C. J. Verissimo[3], *V. R.E.P. Bittencourt*[2], *I. Kinney Ferreira de Miranda Santos*[1]
[1]University of São Paulo, Biochemistry and Immunology, Ribeirão Preto, Brazil
[2]UFRRJ, Department of Animal Parasitology, Seropedica, Brazil
[3]Institute of Animal Science, Nova Odessa, SP, Brazil

Entomopathogenic fungi (EPF) have been widely explored for their potential in biological control as an alternative to pesticides The present study sought to induce tolerance to oxidative stress in EPF and tested it combined with an anti-tick vaccine. Tolerance was induced by successive subculturing with a pro-oxidant agent. CTR (control ticks exposed to water and 0.1% polysorbate 80), CTR-EPF (EPF after subculturing in medium), and EPF-T (ROS-tolerant isolate) were divided into groups, fixed in petri dishes and topically treated with 10 μL EPF at 1 × 108 conidia mL^{-1}. Total weight of egg mass (WEM), larvae hatching percentage (LHP), egg production index (EPI), nutritional index (NI), reproductive efficiency (RE) and tick control (%) were evaluated. After the *in vitro* assay, *Urochloa decumbens* seeds were sown in plastic pots and, after vegetation growth, EPF was distributed on the pots. Engorged female *Rhipicephalus microplus* ticks were collected from bovines previously immunized with a multicompetent anti-tick vaccine containing antigens that target tick innate immunity and also controls, then were distributed on the pots. Eclosed larvae were then recovered from grass apices and quantified. The relative efficacy to control larvae was calculated based on their population density in each pot. The sub-cultured, tolerant strain showed increased antioxidant defenses. In addition to having greater virulence towards tick females, the tolerant EPF showed a significant increase in tick control (84.44%) and 70% reduction in WEM and EPI. For larvae recovery, even though rainfall was low, EPF reduced tick larvae on pots by 30% in the non-vaccinated group. Furthermore, when ticks from vaccinated bovines were exposed to EPF, larvae reduction was ~75%. The present study may open improvements in tick control, replacing harmful chemicals by association between entomopathogens for biological control and vaccines that neutralize tick immunity.

O 057

Deciphering immune pathways involved in cattle tick-resistance by genomic and transcriptomic data

T. E. Galdino Andrade[1], *P. Oliveira*[1], *R. Souza Bin*[1], *M. Priolo Grejo*[1], *M. Scavassini Peña*[1], *J. Fiorotti*[1]
I. Kinney Ferreira de Miranda Santos[1]
[1]University of São Paulo, Biochemistry and Immunology, Ribeirão Preto, Brazil

Controlling tick infestations is relevant for food safety, thus understanding the mechanisms that determine tick loads in cattle is of great importance, mainly to enable effective ways to control this parasite. Tick loads vary between cattle breeds; however, little is known about the immune factors involved in the observed phenotypes. Herein we aimed to identify pathways involved in controlling tick infestations in genetic (natural) and vaccine-induced tick-resistance. Initially, we selected six datasets based on the detection of quantitative trait loci (QTLs) and differentially expressed genes (DEGs) associated with tick loads: two genome-wide association studies (GWAS), one skin transcriptome of Holstein and Nellore, one peripheral blood leukocyte transcriptome of Brangus, one dataset of SNPs in Braford and Hereford, and one blood transcriptome of vaccine-induced resistance in Holstein. Each dataset was analyzed individually using the Metacore platform to find common biological process. A cluster of 127 genes was found overlapping all datasets and included in the Metacore analysis to understand common processes. The main pathways identified in tick-resistant cattle suggests that Neutrophil Extracellular Traps (NET), neuronal sensing, angiogenesis by Ephrin receptors and glutathione metabolism are involved in these animal's phenotype, with a great involvement of MAPK3 cascades. Data suggest that resistant bovines are more sensitive to tick crawling on the skin via MAPK3 and complement pathways, and after the establishment of parasitism, tick saliva containing reactive oxygen species activates MAPK3/p47-phox cascade which is involved in NET release, and intrinsic downregulation of glutathione mediators seems to be enabling this process. MAPK3 also appears to be involved in local leucocyte recruitment in tick-resistant cattle. Further studies should focus on these pathways for the development of technologies to control tick infestations in cattle.

P 055

Immunofluorescence analysis reveals no increased seroprevalence of anti-*Bartonella schoenbuchensis*-IgG antibodies in German forest workers

K. N. Buntrock[1], *W. Ballhorn*[1], *H. Podlich*[1], *J. Malmström*[2], *S. Chowdhury*[2], *K. Hipp*[3], *M. Schaller*[4]
A. Jurke[5], *V. Kempf*[1]
[1]University Hospital Frankfurt, Frankfurt a.M., Germany
[2]Lund University, Division of Infection Medicine, Lund, Sweden
[3]Max Planck Institute for Developmental Biology, Electron Microscopy Facility, Tuebingen, Germany
[4]Eberhard Karl University of Tübingen, Department of Dermatology, Tuebingen, Germany
[5]Landeszentrum Gesundheit Nordrhein-Westfalen, Bochum, Germany

Background: *Bartonella schoenbuchensis* is suspected to cause deer ked dermatitis and febrile diseases in humans. Deer keds (*Lipoptena cervi*) which infest cervids (e.g., roe deer, fallow deer) have been demonstrated to harbour *B. schoenbuchensis* DNA. In terms of a one health perspective, deer keds are discussed as potential vectors for *B. schoenbuchensis*. Methods: We analysed the seroprevalence of anti-*B. schoenbuchensis*-IgG antibodies in sera of forest workers (FW; $n = 82$) compared to control sera of non-forest workers (NFW; n = 118) from North Rhine-Westphalia, Germany. For this purpose, an immunofluorescence assay (IFA) using Vero E6 cells infected with *B. schoenbuchensis* was established, and serum titres were assessed. Immunoblotting using *B. schoenbuchensis* whole cell lysates was performed to identify potential immunodominant target proteins. Results: Using polyclonal rabbit anti-*B. schoenbuchensis*-IgG, the herein established IFA antigen was technically evaluated. When using human sera, 54.9% (n = 45/82) of FW were tested positive at a titre ≥320 whereas IFA reactivity was 66.1% (n = 78/118) in NFW. When the cut-off titre was set to ≥640, then 18,3% (n = 15/82) and 20,3% (n = 24/118) displayed seroreactivity, respectively. In immunoblot analysis, IFA-positive sera reacted with 18 different bands ranging from ca. 40-300 kDa. Conclusions: Our data do not demonstrate elevated seroprevalence of anti-*B. schoenbuchensis*-IgG titres in FW which are regularly exposed to deer keds. Therefore, FW do not seem to have a higher risk to suffer from *B. schoenbuchensis* infections.

P 056

Seroprevalence of vector-borne relapsing fever in Kenya – a retrospective study

M. Olesiuk[1], *F. Reyer*[1], *F. Röttgerding*[1], *D. Waithiru*[1,2], *J. Njeru*[2], *P. Kraiczy*[1]
[1]University Hospital Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt a.M., Germany
[2]Kenya Medical Research Institute, Microbiology, Nairobi, Kenya

Introduction: Relapsing fever (RF) is a vector-borne zoonotic disease transmitted to humans by lice or ticks and is associated with significant morbidity and mortality. Currently, the main focus on louse-borne RF is geographically restricted to Eritrea, Ethiopia, and South-Sudan where outbreaks are frequently reported. Kenya continues to receive increasing number of refugees from these neighboring countries. The current study aimed to assess the seroprevalence of RF among individuals living in areas potentially infested with the vector(s) and patients with a history of recurrent fever.

Methods: Blood and serum samples were collected between 2009 and 2010 from 1,041 patients living in the Turkana county in northern Kenya bordered by Uganda, South Sudan, and Ethiopia. These samples were initially screened for the presence of anti-*Borrelia* IgG antibodies by employing a newly developed ELISA for the serodiagnosis of louse-borne RF. The immunoreactivities of the positive samples were further verified by conducting immunoblots with whole cell lysates of *B. recurrentis.*

Results: Our serological survey revealed that six out of 1.041 (0.6%) of the analyzed patient samples were seropositive and additional 18 samples (1.8%) were just below the threshold (<1.68) in the ELISA. Five out of six patients (four males and two females) tested positive for *Borrelia* antibodies were from Turkana North and were in the age of 28 to 46 years (mean; 39). All six positive samples showed weak to strong IgG immunoreactivities to multiple borrelial proteins of 37-, 45-, 50-, 70-, 90-, and 100 kDa in the whole cell immunoblot and two samples also displayed strong IgM antibody responses.

Conclusion: Taken together, our findings provide evidence for the presences of relapsing fever spirochetes in northern Kenya even with a low frequency of exposure to *Borrelia*. Nevertheless, these data should reinforce public health awareness for a potential outbreak of the disease in northern Kenya.

P 057

Study on ticks for infection with *Borrelia burgdorferi* sensu lato and the impact of two climatic factors

T. Gladnishka[1], *I. Christova*[1], *I. Trifonova*[1], *K. Ngoc*[1], *V. Ivanova*[1], *E. Panayotova*[1], *E. Taseva*[1]
[1]National Centre Of Infectious And Parasitic Diseases, Microbiology, Sofia, Bulgaria

Introduction: Lyme borreliosis is the most common vector-borne illness in almost all over the world. The main vector is *Ixodes ricinus*. The ticks are expanding their range of distribution, according to global warming. The spring precipitations interact with the temperature effects.
The objectives are to investigate infection with *Borrelia burgdorferi* sensu lato in ticks removed from humans and to analyse abundance of ticks in relation to temperature and precipitations in 2022 in Sofia as well as to compare the findings with the data from the previous four years.
Materials & methods: A total of 1,712 ticks were collected and identified in 2022. Nested PCR was performed targeting the spacer region between 5S and 23S of *B. burgdorferi* s.l. rRNA. The meteorological data were received from meteoblue.com. Diagrams were made for distributions of the ticks and for the infection with *B. burgdorferi* s.l. Data on the number of ticks were analyzed against average temperature and precipitations in 2022. The data from 2022 were compared to our data from 2018-2021.
Results: It was found that 95.7% of the ticks belonged to the species *I. ricinus.* The average infestation of the species *I. ricinus* was 19.60%. Nymphs (20.27%) were infected almost at the same rate as the females (20.74%) only in 2022, while they were infected at a higher rate than the females in the other four years. Spring with continuously rising temperatures and a lot of rain led to the highest number of ticks in June 2021.
Conclusion: Temperature and precipitations were found to impact *I. ricinus* abundance. Occurrence of Lyme borreliosis is seasonal and the annual spring onset of cases is mediated by meteorological conditions in preceding weeks. Monitoring of the investigated climatic factors indicate that the number and level of infestation with *I. ricinus* ticks might be taken into account for the risk assessment of Lyme borreliosis.

This work was supported by the project BG05M2OP001-1.002-0001.

P 058

High disease burden of Tick-borne Encephalitis (TBE) virus infection in Latvia despite availability of FSME-IMMUN, a highly effective TBE vaccine, 2018–2020 – Need to increase TBE vaccine uptake

Z. Freimane[1], G. Karelis[2], L. Harper[3], C. Bender[3], P. Zhang[3], A. Pilz[4], F. Angulo[3], W. Erber[4], A. Bormane[5] H. Madhava[6], J. Moisi[7], L. Jodar[3], D. Zavadska[1]
[1]Children's Clinical University Hospital, Riga Stradins University, Department of Pediatrics, Riga, Latvia
[2]Riga East University Hospital, Department of Neurology and Neurosurgery, Riga, Latvia
[3]Pfizer Vaccines, Medical and Scientific/Clinical Affairs, Collegeville, PA, United States
[4]Pfizer Corporation Austria, Pfizer Vaccines, Vienna, Austria
[5]Centre of Disease Prevention and Control, Infectious Diseases Surveillance and Immunization Unit, Riga, Latvia
[6]Pfizer Vaccines, Medical and Scientific/Clinical Affairs, London, United Kingdom
[7]Medical and Scientific/Clinical Affairs, Pfizer Vaccines, Paris, France

Background: Tickborne encephalitis (TBE), an infection by the tickborne encephalitis virus (TBEV) resulting in signs or symptoms of central nervous system (CNS) inflammation, can result in severe clinical consequences including death. TBE is endemic in parts of Europe and Asia. Despite availability of FSME-IMMUN, a vaccine that is >99% effective in preventing TBE, hundreds of TBE cases occur yearly in Latvia.

Methods: Rīga Stradinš University conducted enhanced surveillance for TBEV infections in collaboration with the Centre for Disease Prevention and Control of Latvia, the National Reference Laboratory, and the 15 hospitals where TBE cases are hospitalized. Serum and cerebrospinal fluid (CSF) were tested by ELISA for TBEV-specific IgG and IgM antibodies. A TBE case was defined as a laboratory-confirmed TBEV-infected patient with CNS signs or symptoms. After informed consent, vaccine history and clinical course of TBE was collected by medical record review and patient interview.

Results: From 2018-2020, 587 TBE cases were identified, of which 579 (99%) were hospitalized. Among TBE cases with a known vaccine history, 99% (576/584) reported never receiving a TBE vaccine. The population-based incidence of TBE was 10.3 cases/100,000 population (range by region 2.3-36.8/1000) per year. Among the 579 hospitalized TBE cases, 17 (3%) were admitted to ICU and 13 (2%) died. Median length of hospitalization of TBE cases was 11 days (interquartile range 9-14 days) and 21 (4%) TBE cases were discharged with paresis. Of the 13 TBE patients that died, 12 (92%) were unvaccinated and 1 (8%) had an unknown vaccine history.

Conclusion: The high burden of TBE in Latvia, which occurs almost entirely in the unvaccinated population, illuminates a problem of inadequate nationwide TBE vaccine uptake. Increased public health efforts are needed to prevent life-threatening TBE, included enhanced efforts to increase TBE vaccine uptake in Latvia and all European regions where it is endemic.

P 059

Metatranscriptomics uncovers diverse tick-borne bacteria, protozoa, and viruses in Australian ticks

A. Gofton[1]
[1]CSIRO, Health and Biosecurity, Canberra, Australia

Question: Ticks can harbour a diverse range of pathogens, from obligate intracellular bacteria, extracellular bacteria, protozoan blood parasites, and a wide range of viruses. Metatranscriptomics is a promising tool to audit this complex pathobiome, however, many researchers use this technology to investigate viruses only, neglecting valuable insights into other pathogen groups. We aimed to use metatranscriptomics to audit the complete range of bacterial, protozoan, and viral pathogens in two sympatric tick species with different host preferences.
Methods: Questing *Ixodes holocyclus* and *Haemaphysalis bancrofti* were collected from two sites in eastern Australia, and in total 3,665 ticks were grouped into 178 pools. Bulk DNA and RNA was extracted and sequenced on Illumina platforms. A custom metatranscriptomic bioinformatics pipeline was used to identify tick-borne pathogens and determine microbiome community composition. Following metatranscriptomic analysis PCR and Sanger sequencing was used to confirm the identity of novel tick-borne microorganisms.
Results: Metatranscriptomics provided a wholistic audit of the pathobiome of two tick species and was able to identify a wide range of tick-borne pathogens in a single analysis, including bacteria such as *Anaplasma, Ehrlichia, Neoehrlichia*, *Rickettsia*, and *Borrelia*, protozoa such as *Babesia, Theileria*, *Hepatozoon,* and *Trypanosoma,* and viruses including Reoviridae, Flaviviridae, Rhabdoviridae, and Iflaviridae. In total our analysis identified 32 tick-borne pathogens including 10 novel tick-borne taxa, which included novel *Borrelia*, *Trypanosomes,* and coltiviruses.
Conclusions: Metatranscriptomics is an efficient way to audit ticks' complete pathobiome and provide insights into novel pathogen species, strains, and the microbial community as a whole. Here we have shown the utility of this technology and uncovered a remarkable diversity of vertebrate-infecting tick-borne microorganisms in two sympatric ticks.

P 060

Variant sequence analyses of *Anaplasma phagocytophilum* for *16S* rRNA and *msp4* in positive dog and horse samples from Germany

S. Fischer[1]*, I. Schäfer*[2]*, B. Kohn*[3]*, C. Weingart*[3]*, C. Silaghi*[1]*, E. Müller*[2]
[1]Friedrich-Loeffler-Institute, Institute of Infectology, Greifswald, Germany
[2]LABOKLIN GmbH and Co. KG, Bad Kissingen, Germany
[3]Clinic for Small Animals, Freie Universität Berlin, Department of Veterinary Medicine, Berlin, Germany

Introduction: *Anaplasma* (*A.*) *phagocytophilum* is a tick-transmitted bacterium causing granulocytic anaplasmosis. This pathogen shows a high variability of different genetic variants even within individual host species.

Objectives

The aim of this study was to unravel the variant diversity from dogs and horses in Germany.

Methods: Left-over DNA samples from dogs and horses tested positive for *A. phagocytophilum* by PCR were provided by the laboratory LABOKLIN. Hematological data and epidemiological metadata were available. DNA was extracted and samples were screened positive by qPCR, followed by nested PCR for detection of a 546-bp fragment of *16S* rRNA and a 362-bp fragment of the *msp4* gene, respectively. Sequences of these products were generated by Sanger-Sequencing.

Results: In total, 46 canine and 36 equine samples were included in the variant analyses. The main variant detected in dogs and horses for *msp4* was the variant formally known as m4-20 reported in hosts like dogs, sheep, and hedgehogs. Additionally, in 3 dogs, a new *msp4* variant was detected and two horses showed relation to a formally known variant (m4-18) and to a described variant found in sika deer (m4-47), respectively. For *16S* rRNA, the variant 16S-2 was expectedly detected in dogs and was also found in horses. In addition, 13 of the dog samples displayed variant 16S-1.

Conclusions: The results describe a distribution of variants of *A. phagocytophilum* formerly not known. Some variants seem to correlate with none or mild hematological scores which may result in pathogen reservoirs and may contribute to the spread of the pathogen. Further investigations are needed for confirmation.

P 061

Effect of forest structure and landscape parameters on *Ixodes ricinus* tick abundance and infectivity

K. Kybicová[1], *Z. Vacek*[2], *J. Cukor*[2], *E. Richtrová*[1], *S. Vacek*[2], *T. Václavík*[3]
[1]National Institute of Public Health, National Reference Laboratory for Lyme Borreliosis, Prague, Czech Republic
[2]Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Prague, Czech Republic
[3]Palacký University Olomouc, Faculty of Sciences, Olomouc, Czech Republic

Ixodes ricinus is the most widespread vector of tick-borne diseases in Europe. In recent years, we observed an increased abundance of ticks and their expansion into higher altitudes due to climate changes. Locally, the geographical distribution of ticks is also influenced by forest and landscape parameters.

This work aims to determine the relationship between the forest structure and landscape parameters on *I. ricinus* abundance and infectivity in various forests in Czechia. We collected 13,645 ticks in 2021 from 150 forest areas; 13,632 ticks were *I. ricinus*, 5 were *Dermacentor reticulatus*, and 8 were *Haemaphysalis concinna*. *I. ricinus* nymphs from 68 representative plots were tested using PCR for the presence of *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum*.

The density reached 1.29 ticks per 10 m^2. The highest *I. ricinus* abundance was observed in coniferous stands and forest edges with a high incidence of wild ungulates. Forest fragmentation also had a significantly positive effect on tick abundance. Contrarily, the lowest tick numbers were observed in clear-cut biotopes, mixed stands, and large forest complexes. Increasing vertical stand structure as well as tree species diversity and a distance from a habitation had a significantly negative effect on tick abundance. Ticks were infected by *B. burgdorferi* s.l. in 10.4%. The borrelia infection increased with elevation, forest fragmentation, and with a decreasing occurrence of ungulates. *A. phagocytophilum* infection was detected in 1.8% of ticks. This infection was positively correlated with tick abundance and negatively with borrelia infection.

Based on the accumulated knowledge, it should be possible to predict the *I. ricinus* abundance in the changing conditions of Central Europe. We assume that silviculture of mixed forests, which are resistant to climate extremes, could reduce the tick abundance, and consequently also the presence of tick-borne diseases compared to receding coniferous monocultures.

P 062

Identification of Lyme neuroborreliosis outside of Hokkaido, Japan

A. Takano[1]*, M. Ohira*[2]*, N. Maeda*[3]*, H. Nakajima*[4]*, A. Arai*[5]*, Y. Kobayashi*[6]*, L. Harper*[7]*, J. Edwards*[7]
C. Bender[7]*, A. Pilz*[8]*, K. Wada*[6]*, F. Angulo*[7]*, W. Erber*[8]*, J. Stark*[7]*, J. Moisi*[9]*, L. Jodar*[7]*, M. Takao*[2]
[1]Yamaguchi University, Laboratory of Veterinary Epidemiology, Yamaguchi, Japan
[2]National Center of Neurology and Psychiatry, Department of Clinical Laboratory, Tokyo, Japan
[3]National Hospital Organization Beppu Medical Center, Department of Neurology, Beppu, Japan
[4]Nihon University Itabashi Hospital, Department of Neurology, Tokyo, Japan
[5]Aomori Prefectural Central Hospital, Department of Neurology, Aomori City, Japan
[6]Pfizer Vaccines, Japan Medical Affairs, Tokyo, Japan
[7]Pfizer Vaccines, Medical and Scientific/Clinical Affairs, Collegeville, PA, United States
[8]Pfizer Corporation Austria, Pfizer Vaccines, Vienna, Austria
[9]Medical and Scientific/Clinical Affairs, Pfizer Vaccines, Paris, France

Background: Lyme borreliosis (LB), a tick-borne disease caused by *Borrelia burgdorferi* sensu lato, commonly results in erythema migrans but can result in disseminated disease including Lyme neuroborreliosis (LNB). LB is a nationally notifiable disease in Japan with 10-20 LB cases mostly from Hokkaido, but LNB is rarely reported. LB and LNB are likely under-diagnosed in Japan since LB diagnostic tests are not readily domestically available.
Methods: Serum and cerebrospinal fluid (CSF) collected in 2010-2021 from hospitalized patients >1 year-of-age with encephalitis or meningitis of unknown etiology hospitalized at 15 hospitals were tested for *B. burgdorferi* sensu lato antibodies using an in-house ELISA and confirmatory RecomLine Blot (MIKROGEN). A confirmed LNB case was a patient with CSF pleocytosis and evidence of intrathecal production of Lyme borreliosis antibodies; a probable LNB case was a patient with CSF pleocytosis and positive Lyme borreliosis serology in CSF. Patient information was collected from medical records.
Results: Residual specimens were available from 502 patients. Three patients hospitalized with meningitis were identified as a confirmed or probable LNB case. The confirmed LNB case was a male Aomori City (Aomori prefecture) resident aged 38 years hospitalized for 9 days in February 2021. The two probable LNB cases were a female Asaka City (Saitama) resident aged 26 years hospitalized for 17 days in April 2017 and a female Beppu (Oita) resident aged 35 years hospitalized for 4 days in May 2019. None of the three patients had specimens previously tested for LB or reported travel, tick bite, or exposure to tick habitat in the month prior to symptom onset.
Conclusion: Lyme neuroborreliosis was identified outside of Hokkaido, Japan. The identification of previously undiagnosed LNB demonstrated the potential for other undiagnosed LB cases in Japan and indicates a need for increased disease awareness, clinical testing, and prevention efforts.

P 063

Building the generic roadmap for “Vector Transmission Control”

B. M. Goddeeris[1,2], I. Kinney Ferreira de Miranda Santos[3], M. Newman[4]
[1]Catholic University of Leuven, Animal Health, Leuven, Belgium
[2]University of Ghent, Department of Parasitology, Immunology, Virology, Ghent, Belgium
[3]University of São Paulo, Biochemistry and Immunology, São Paulo, Brazil
[4]Secretariat to STAR-IDAZ IRC, Department of Environment, Food and Rural Affairs, London, United Kingdom

The Strategic Alliance for Research on the Major Infectious Diseases of Animals and Zoonoses (STAR-IDAZ) International Research Consortium (IRC) is a global initiative to coordinate global animal health research at international level to accelerate delivery of new and improved animal health strategies and disease control tools. To achieve these goals, the IRC partners agree to coordinate their research programmes to address identified research needs relating to the various topics and to share results. Research gaps identified by the Working Groups are organized into research roadmaps, which map out the key research questions needing to be addressed, working from a target product profile back to basic science. The roadmaps are based on steps or “Leads” which each consider the Research Question (“What is the problem needing to be solved?”), Challenges (“What are the scientific and technological challenges/knowledge gaps needing to be addressed”), Solution Routes (“What approaches could/should be taken?”), Dependencies (“What needs to be done first?”) and State of the Art (Existing knowledge including success and failures) (Entrican et al. 2020). One of the STAR-IDAZ priority topics is “Vector Transmission Control” (VTC), in addition to several vector-borne diseases. The generic roadmap for VTC can then be used in conjunction with STAR-IDAZ roadmaps specific to vector-transmitted diseases. A draft roadmap for VTC has already been constructed and aligned on three routes, i.e. vector biology, vector host and vector biotope, but each building block of this routes has now to be complemented by state-of-the-art information from experts on the relevant Leads to ensure that the necessary research gaps are addressed for identification and design of relevant projects.

Entrican et al., 2020. Transbound. Emerg. Dis. 68: 1513-1520.

P 064

Importance of individual reference values in Lyme antibody testing

E. Volokhina[1], A. Smits[1], Y. Smits[1], J. de Groote[1], J. Lankhof[1], W. Anker[1], S. Theunisse[1],
K. Plaum[1], M. Kouwijzer[1], A. Garritsen[1]
[1]Innatoss Laboratories, Oss, Netherlands

Introduction: Success of therapy for Lyme borreliosis is higher in the early phases of disease, therefore timely diagnosis is crucial. Inability to distinguish between a past and ongoing infection is an important drawback of antibody testing. Our laboratory performs yearly Lyme screening for individuals with high occupational exposure to ticks.

Objectives: To increase the utility of Lyme antibody screening, we developed a new approach where antibody levels are compared to a reference sample from the same person acquired before the tick season, rather than to the normal range established by a test manufacturer.
Materials and Methods: In 2021, 3759 individuals from all 12 Dutch provinces participated in the screening. *Borrelia* IgG and IgM antibodies were measured by ELISA (Euroimmune and Zeus), positivity was confirmed by immunoblot (Viramed). Individuals with more antibodies than in the reference sample were advised to seek medical attention and received an invitation for the follow up measurement at least three months later.
Results: In 654 individuals *Borrelia* antibodies were above the manufacturer's normal range. However, only in 187 (29%) of them, the antibody levels were increased in comparison to the reference sample. 115 individuals participated in the follow up measurement. At follow up, only 20 (17%) returned to negative values and the positive values varied greatly.
Conclusion: Only one third of antibody levels above the manufacturer's normal range was caused by infection in the most recent season in 2021. Once increased, the antibody levels remained high in most of individuals for at least three months. Establishing individual reference values for participants increases utility of Lyme antibody screening for detection of a new infection.

P 065

Detection of *Babesia canis* in *Dermacentor reticulatus* from Germany

A. Springer[1], *A. Lindau*[2], *J. Probst*[1], *K. Fachet*[2], *G. Dobler*[3], *U. Mackenstedt*[2], *C. Strube*[1]
[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hannover, Germany
[2]University of Hohenheim, Institute of Biology, Department of Parasitology, Stuttgart, Germany
[3]Bundeswehr Institute of Microbiology, Munich, Germany

Introduction: The ornate dog tick *Dermacentor reticulatus*, vector of *Babesia canis*, has shown a marked range expansion in Europe and is now present all over Germany. Previously, only few areas in Germany were recognized as endemic for canine babesiosis, a potentially fatal disease of dogs, however, the incidence seems to be increasing.
Objectives: To better assess the current risk for dogs, the objective of the present study was to determine the prevalence of *B. canis* in *D. reticulatus* specimens collected from all over Germany.
Material and Methods: In the frame of a citizen science study, *D. reticulatus* specimens collected during the years 2019-2022 were obtained from all over Germany. Individual ticks were subjected to *Babesia* detection via quantitative real-time PCR. Positive samples were confirmed by conventional PCR and sequencing of the *Babesia* 18S rRNA gene.
Results: Among 3,411 tested specimens yet, two were *B. canis*-positive (0.06%). Both of these ticks were discovered on dogs and likely originated from the area of Freiburg im Breisgau, federal state of Baden-Wuerttemberg, which is known to be endemic for canine babesiosis. However, one of the ticks was detected on a dog in Fürth, federal state of Bavaria, after returning from a trip to the Breisgau on the previous day.

Conclusion: The overall prevalence of *B. canis* in the German *D. reticulatus* population seems to be low, as the pathogen is still restricted to endemic foci. However, this study shows that travelling dogs contribute to spreading of the pathogen to non-endemic areas.

P 066

Seasonal variation of *Neoehrlichia mikurensis*, *Borrelia burgdorferi* s.l. and TBEV related to the effect of climate change in Mandal, Norway.

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

Introduction: In Norway, two pathogenic bacteria species are the most prevalent in *Ixodes ricinus* ticks, *Borrelia burgdorferi* s.l. and *Neoehrlicha mikurensis*. The prevalence and transmissibility of tick-borne pathogens are affected by external biotic and abiotic factors since ticks spend most of their lives off-host. Climate change alters the ticks' distribution, abundance, survival, reproduction, and questing behavior, as well as the availability of hosts. It is essential to evaluate patterns at low geographic scales within the distribution since differences in the climatic preference of this tick have been observed in different regions of Europe.

Objective: The principal aim is to evaluate the prevalence variations of *B. burgdorferi* s.l. and *N. mikurensis* in nymphs during the tick season from April to November of 2020 at a single location in Southern Norway (Kilen, Mandal) based on a climatological frame.

Methods: 200 samples per month are being analyzed through RT-PCR. Statistical tests will be employed to estimate significant differences between each month and pathogen prevalence. Finally, micro and macroclimatic parameters from the local station equipment and from the Norwegian Meteorological, in addition to results from the CORDEX project will be used to predict the future scenarios in different regional and global climatic models.

Results: Thus far, 833 samples have been analyzed for *N. mikurensis*, giving monthly prevalence from 4% to 9% from April to November. For the case of *Borrelia burgdorferi* s.l. 255 samples have been analyzed, with a range of prevalence from 12% to 24% for June, July, and September.

Conclusions: These early results show that *N. mikurensis* was present in ticks for the entire questing season of 2020 in that region, with prevalence values comparable to previous studies in southern Norway, which report prevalence between 5 and 11% in nymphs, as well as *B. burgdorferi* s.l., where prior research reports prevalences from 10 to 25%.

P 067

Outdoor worker knowledge on ticks and Lyme disease across demographics

E. Fellin[1], *V. Millien*[1], *M. Varin*[2]
[1]McGill University, Biology, Montreal, Canada
[2]CERFO, Quebec City, Canada

Lyme disease is a well-known occupational risk across North America due to the exposure of the causal agent of Lyme disease (*Borrelia burgdorferi*) via blacklegged ticks (*Ixodes scapularis*). As the geographic range of *B. burgdorferi* advances with the increasing distribution of blacklegged ticks, individuals working at outdoor sites are more at risk of contracting Lyme disease. In this study, we analyzed the demography and personal preventative behaviours within one framework to better determine the overall risk for those working in outdoor industries. It was expected that individuals who spend more time outdoors would be more at risk, since they are exposed to the environment longer. By analyzing outdoor worker demographics and individual knowledge on ticks and Lyme disease, a comparison was made across age, sex, education, and industry as well as time spent outdoors (during work and recreationally). A questionnaire was given to voluntary participants where risk scores were developed based on individuals' answers to risk perception questions. Two risk scores were developed; one considering behaviours of individuals, while the other took into account geographical risk. Analyzing demographic and time spent outdoors predictors for these risk scores via ANOVAs, we found that there were no significant demographic effects on an individual's behaviour risk score. However, those who are more experienced outdoors are likely to have lower scores. examining and acknowledging the specific vulnerabilities that individuals have while working, will allow for better management and awareness programs to be put in place.

P 068

Diversity of ticks associated with wild animals in Cameroon

A. Paguem[1,2], *M. Kingsley*[2,3], *P. Kamtsap*[1], *A. Renz*[4], *S. Schaper*[4], *G. Dobler*[4,5], *D. K. Bakkes*[6]
L. Chitimia-Dobler[4]
[1]University of Tübingen, Institute of Evolution and Ecology, Tübingen, Germany
[2]Faculty of Agriculture and Veterinary Medicine, The University of Buea, Buea, Cameroon
[3]Bangangte Multipurpose Research Station, Bangangte, Cameroon
[4]Bundeswehr Institute of Microbiology, Munich, Germany
[5]University of Hohenheim, Zoology, Stuttgart, Germany
[6]Agricultural Research Council, Gertrud Theiler Tick Museum, Onderstepoort Veterinary Research, Pretoria, South Africa

Ticks are obligate blood-sucking parasites of wild animals and transmit many zoonotic microorganisms which can spread to domesticated animals and then to humans. In Cameroon, little is known about tick diversity among wildlife, especially animals which are hunted for human consumption. Therefore,

this survey was undertaken to investigate tick species diversity parasitizing wild animals sold in bush meat markets in Cameroon. In total, 686 ticks were collected and identified to species level based on morphology, and some were genetically analyzed using the *16S* rDNA gene. Eighteen tick species belonging to five genera were identified: *Amblyomma* spp. (*Amblyomma compressum*, *Amblyomma flavomaculatum*, *Amblyomma variegatum*), *Haemaphysalis* spp. (*Haemaphysalis camicasi*, *Haemaphysalis houyi*, *Haemaphysalis leachi*, *Haemaphysalis parmata*), *Hyalomma* spp. (*Hyalomma nitidum*, *Hyalomma rufipes*, *Hyalomma truncatum*), *Ixodes* spp. (*Ixodes rasus*, *Ixodes moreli*), and *Rhipicephalus* spp. (*Rhipicephalus guilhoni*, *Rhipicephalus moucheti*, *Rhipicephalus muhsamae*, *Rhipicephalus microplus*, *Rhipicephalus camicasi*, *Rhipicephalus linnaei*). In terms of microorganisms important for public health, two *Rickettsia* spp., namely *Rickettsia aeschlimannii* and *Rickettsia africae*, were detected in *Hyalomma* spp. and *Amblyomma* spp., respectively. Furthermore, *Candidatus* Rickettsia africaustralis was detected for the first time in Cameroon in *I. rasus*. This study highlights the high diversity of ticks among wildlife sold in bush meat markets in Cameroon.

P 069

CCHFV and RVFV antibodies in cattle from Mozambique – closing a knowledge gap in southern Africa

J. Fafetine[1,2], T. Cuinhane[1], R. Miambo[1], B. Sadeghi[3], L. de Araújo[2], M. H. Groschup[3], A. Schulz[3]
[1]Universidade Eduardo Mondlane (UEM), Faculdade de Veterinária, Maputo, Mozambique
[2]Universidade Eduardo Mondlane (UEM), Centro de Biotecnologia, Maputo, Mozambique
[3]Friedrich-Loeffler-Institute, INNT, Greifswald, Germany

Arboviruses increasingly pose a threat to public health in sub-Saharan Africa countries. Mozambique is located in a region that is prone to climate change-related devastation, including heavy rainfalls and severe droughts that favor the emergence of zoonotic viruses transmitted by arthropods such as Crimean-Congo hemorrhagic fever virus (CCHFV) and Rift Valley fever virus (RVFV). Available previous data on the occurrence of RVFV indicate that the disease occurs in all the sampled provinces. However, data related to the presence of CCHFV in the country is rather scarce. Hence, the objective of this study was to evaluate the recent seroprevalence of CCHFV and RVFV in cattle in four localities of Limpopo National Park. A total of 460 cattle blood samples were collected and analyzed for the presence of anti-CCHFV and anti-RVFV antibodies using ID Screen CCHF Double Antigen Multi-species (IgG) and ID Screen Rift Valley Fever Competition Multi-species commercial ELISA test kits (IDvet, Grabels, France), respectively. Additionally, 1176 ticks were collected from the same animals and analyzed with different qRT-PCRs assays for CCHFV, Nairobi sheep disease virus (NSDV) virus and Dugbe virus (DUGV). Selected ticks were further screened by using a pan-Flavivirus PCR. The overall seroprevalence was higher for CCHF (50 %) than for RVF (28 %). Neither RT-qPCR positive samples nor specific BLAST results for Flaviviruses were obtained for the analyzed ticks. This study revealed the presence of anti-CCHFV and anti-RVFV antibodies in cattle from all the four sampled localities suggesting that both viruses are circulating in cattle and may be an important cause of unidentified febrile illness in humans in that region.

P 070

Molecular detection of zoonotic bacteria in ticks parasitizing cats

M. Razgūnaitė[1], J. Radzijevskaja[1], I. Lipatova[1], V. Razgūnė[2], A. Paulauskas[1]
[1]Vytautas Magnus University, Biology, Kaunas, Lithuania
[2]Kaunas University of Applied Sciences, Medicine, Kaunas, Lithuania

Ticks, infesting cats, have rarely been examined for the presence of vector-borne zoonotic pathogens. Parasitic arthropods (ticks and fleas), associated with companion animals that have close contact with humans, can potentially transmit zoonotic vector-borne pathogens such as *Rickettsia* spp. and *Mycoplasma* spp. This study aimed to evaluate the prevalence of *Rickettsia* spp. and *Mycoplasma* spp. in ticks collected from domestic cats in Lithuania. A total of 321 ticks belonging to two species, *Ixodes ricinus* and *Dermacentor reticulatus*, were collected from 59 infested cats from various regions of Lithuania. Conventional PCR assay amplifying partial 16s rRNA gene was used for the detection of *Mycoplasma* spp., and nested PCR assays amplifying *gltA*, and *17-kDa* genes were performed for the detection of *Rickettsia* spp. The minimum infection rate (MIR) of *Rickettsia* spp. in ticks calculated based on the amplification of two genes was 31.8% (102/321). The MIR of *Rickettsia* spp. in *D. reticulatus* was significantly higher (41.8%) than that detected in *I. ricinus* (29%) ticks. Human pathogenic *Rickettsia helvetica, Rickettsia raoultii* and *Rickettsia monacensis* were identified after sequencing the positive samples. *D. reticulatus* ticks harboured *R. raoultii* and *R. helvetica*, while *I. ricinus R. helvetica* and R. *monacensis.* After amplifying the 16s rRNA gene, one *I. ricinus* sample was found positive. Sequence analysis did not detect *Mycoplasma* spp., but identified the tick intracellular bacterium *Rickettsiella* sp. An obtained sequence of *Rickettsiella* showed 100% identity with *Rickettsiella* sp. detected from *Ixodes uriae* in Iceland. Our data provide new information on the circulation of vector-borne rickettsial pathogens in ticks infesting cats which may represent a potential risk to animal and human health. This study is the first report of emerging bacteria from the genus *Rickettsiella* in *I. ricinus* ticks in Lithuania.

P 071

Microbiota of the brown dog tick *Rhipicephalus sanguineus* s.l. (Acari: Ixodidae) from Israel

G. Arslan Akveran[1], S. Akdogdu[1], D. Karasartova[1], K. Y. Mumcuoglu[2], A. Taylan Ozkan[3]
[1]Hitit University, Corum, Turkey
[2]The Hebrew University-Hadassah Medical School, Microbiology and Molecular Genetics, Jerusalem, Israel
[3]TOBB University, Medical Microbiology, Ankara, Turkey

Introduction: *Rhipicephalus sanguineus* s.l. shows a worldwide distribution and is a known vector of pathogens to humans and animals. The diversity of microbial communities associated with different populations of this species complex remains poorly characterized. Specimens of *R. sanguineus* s.l. in Israel belong to the “Temperate” lineage. Tick microbiota can contribute to vector development, reproduction survival, and competence.

Objectives. The aim of the study was to reveal the relative abundance of bacterial taxa in this tick collected in the region and to demonstrate the presence of potential pathogenic species for its hosts and humans.
Materials and Methods: Specimens of *R. sanguineus* s.l. were collected from domestic dogs (*Canis familiaris*) and Southern white-breasted hedgehogs (*Erinaceus concolor*) in Israel and were analysed using next generation sequencing. Tick pools with 10 adult specimens each were analysed through 16S rRNA V3–V4 region amplification in a MiSeq Illumina, using EzBioCloud as a taxonomical reference.
Results. The operational taxonomic units (OTUs) revealed 34 phyla, 63 classes, 134 orders, 219 families, 403 genera, and 1,391 species. The most prevalent phyla were Proteobacteria, Firmicutes, Actinobacteria, Bacteroidota, and Verrucomicrobia. The most abundant species, *Coxiella* spp. are known endosymbionts of ticks. As potential pathogenic microorganisms for the ticks and humans, representatives of the Yersiniaceae family as well as *Mycoplasma* spp., *Haemophilus* spp., *Mycobacterium* spp., and *Helicobacter* spp. were identified.
Conclusions: The high number of taxa registered for *R. sanguineus* s.l. may be the result of the variety of hosts which these ticks parasitize. Studies on components of the tick microbiota are important in terms of understanding the host-microbe dynamics and potential risks of ticks for known and unknown tick-borne diseases.

P 072

Enumeration of Cytokine secreting cells for the diagnosis of Lyme disease

K. Lukas[1], J. Köstler[1], A. Gessner[1]
[1]University Hospital Regensburg, Institute for Microbiology and Hygiene, Regensburg, Germany

The available diagnostic methods for Lyme disease are still not optimal. In order to close this gap by means of an additional method, our focus is on the identification of borreliosis via the analysis of cytokine secreting cells using the sensitive ELISpot assay. Therefore, the current techniques were initially extended and modified so that a total of nine different functional cytokine ELISpots were available: IFN-γ, IL-4, IL-6, IL-9, IL-10, IL-17, IL-21, IL-22, CXCL13 ELISpots.
We chose five suitable Borrelia specific antigens, four recombinant *Borrelia afzelii* surface antigens (OspA, VlsE, DbpA, p100) and Borrelia lysate from *Borrelia burgdorferi* s.s. for the Lyme disease specific tests.
By using these specific ELISpots, PBMCs of 44 recruited persons, 30 probands with Lyme disease background (patients with erythema migrans, Lyme arthritis, neuroborreliosis and Lyme disease patients with several same-time symptoms) and 14 without it (without anti-Borrelia antigen IgG and IgM and known tick bites), were compared. The ELISpot versions tested did not reveal strong significant differences in the number of spots detected in the control subjects and Lyme disease patients, with the exception of the IL-21 ELISpots. In these, strong significant differences were detectable between erythema migrans and neuroborreliosis patients. On closer examination of this result with the inclusion of additional (follow-up) blood samples, revealed that the IL-21 positive subjects were exclusively patients with severe symptoms. Subjects with weak or no symptoms were negative without exception.

This possible clear assignment of Lyme disease in the case of non-pathognomic but Lyme disease associated severe symptoms via IL-21 ELISpots is a promising approach in Lyme disease diagnostics and thus also for the correct and successful treatment of patients.

P 073

The tick-host interface researched through the comparative in vivo and ex vivo feeding of *Ixodes ricinus* ticks

V. Urbanová[1], T. Hatalová[1], J. Ribeiro[2], P. Kopáček[1], R. Sima[1], J. Perner[1]
[1]Biology Centre of the Czech Academy of Sciences, Parasitology, Ceske Budejovice, Czech Republic
[2]NIH, NIAID, Bethesda, MD, United States

Introduction: Tick midgut cells and tick saliva comes in direct contact with host molecules, representing two distinct Tick-Host Interfaces (THI). The functional association between tick and host molecules at the THI is determined by native quantities of interacting molecules.
Objectives: The diversity of tick saliva components or the function of its constituents has been intensely researched *in vitro*. These assays often overestimate the physiological level of binding partners. Here, we set out to explore the systemic functions of salivary constituents in the genetic or biochemical modulated tick individuals.
Materials & methods:To increase our understanding of the function of tick saliva or its individual components from a tick perspective, we deploy RNAi or administration of small molecule inhibitors and phenotype ticks during in vivo feeding compared to *ex vivo* feeding in a membrane feeding system. Phenotypic display only in the *in vivo* feeding ticks clearly indicates the participation of the studied protein at the THI. Using such a comparison, assignment of individual host immune components, absent in *ex vivo* feeding system, with a function and link to tick feeding molecular physiology can be given.
Results: Results will be presented as “Questions Answered” and these will include: What is the molarity of proteins in tick saliva? What is the size distribution of salivary proteins? Does microsurgery removing salivary glands of *Ixodes ricinus* females affect similarly the feeding performance of ticks *in vitro* and *ex vivo*? Do *I. ricinus* ticks need to fully salivate for *Borrelia afzelii* to get transmitted to a mouse model? Do tick histamine-binding proteins get complexed with histamine of host or tick origin? Is host histamine a stimulant or a suppressant of tick feeding?
Conclusion: Nothing in tick-host biology makes sense except in the light of native molecular quantity.

References: PMID:33312963 PMID: 35167765

P 074

Danger comes from a tree? – Pathogens detected in *Ixodes ricinus* feeding on red squirrels (*Sciurus vulgaris*) from parks in Warsaw

D. Dwużnik-Szarek[1], *A. Beliniak*[2], *W. Malaszewicz*[1], *D. Gryz-Krauze*[2], *J. Gryz*[3], *K. D. Jasińska*[2]
A. Bajer[1]
[1] University of Warsaw, Department of Eco-Epidemiology of Parasitic Diseases, Warsaw, Poland
[2] University of Warsaw, Forestry Faculty of Warsaw University of Life Sciences, Warsaw, Poland
[3]Forest Research Institute, Department of Forest Ecology, Raszyn, Poland

Introduction: *Ixodes ricinus* is the main vector of various pathogens of medical and veterinary significance. One of the important hosts for *I. ricinus* is the European red squirrel, however, no studies on ticks and tick-borne pathogens have been conducted on squirrels in Poland.

Objective: The aims of the study were: comparing tick prevalence and mean infestation between red squirrels trapped in two park sites (Rezerwat Natolin and Łazienki Królewskie Park, Warsaw) and determining the prevalence of the three most common pathogens vectored by *I. ricinus* (*Borrelia*, *Rickettsia* and *Babesia* spp.)

Materials & methods: Red squirrels were live-trapped in 2019 and 2020 from March to September. Prevalence and mean tick infestation depending on year, month and site of rodent trapping were calculated. Ticks were collected, identified and subjected to DNA extraction. PCR reactions were performed using specific primers and reaction conditions (for *Rickettsia*: fragment of the citrate synthase *gltA* (750 bp) for *Borrelia burgdorferi* s.l.: fragment of flagellin (*flaB*); for *Babesia*: fragment of 18S rRNA gene).

Results: In total, 198 red squirrels were inspected for tick infestation. Differences between prevalence and mean tick infestation were observed between squirrels trapped from two locations. Three species of the *B. burgdorferi* complex, *R. helvetica, R. raoultii, R. monacensis* and *B. microti* were detected in collected ticks. Also, pathogens presence differed depending on year, month and host trapping sites.

Conclusions: The obtained results contribute to expanding the knowledge of *Sciurus vulgaris* as an important host for *I. ricinus* and pathogens vectored by common ticks.

Acknowledgements: The study was financially supported by the Ministry of Science and Higher Education through the Faculty of Biology, University of Warsaw intramural grant 501-D114-01-1141000

P 075

Detection of *Phenuiviridae, Chuiviridae m*embers and a novel *Quaranjavirus* in hard ticks from Danube Delta

B. Bratuleanu[1,2], S. Temmam[1], S. Munier[1], D. Chrétien[1], T. Bigot[3], S. van der Werf[1], G. Savuta[2]
M. Eloit[1], A. Bennouna[4]
[1] Pasteur Institute, Virology, Paris, France
[2]Iasi University of Life Sciences, Rovetemerg center, Iasi, Romania
[3] Pasteur Institute, Biostatistic Hub – Computational Biology Department, Paris, France
[4] Pasteur Institute, virology, Paris, France

Introduction: Ticks are involved in the transmission of various pathogens, and several tick-borne diseases cause significant concerns for the health of humans and livestock. The rapid development of next generation sequencing methods led to the identification of many novel viral sequences in ticks of different species from different regions of the world. Among them, members of the Quaranjavirus genus are mainly associated with *Argas* ticks but recent studies demonstrated their presence in ixodid ticks.

Objectives: The objective of this work was to identify viruses associated with *Rhipicephalus* sp. ticks collected from small ruminants in Southern Romania, extending our knowledge on viruses circulating in ticks and the potential risk for ruminants and humans living in the area.

Materials & methods: In 2020, 169 *Rhipicephalus* sp. ticks were collected from small ruminants from Danube Delta region and sequencing was carried out on an Illumina NextSeq500 sequencer in a single-read 1 x 150 bp format. BioIT was conducted with Microseek, an in-house pipeline.

Results: We identified sequences from *Phenuiviridae* and *Chuviridae* families and a new quaranjavirus-like, tentatively named Cataloi tick quaranjavirus (CTQV). Phylogenetic analyses performed on the five segments show that CTQV is phylogenetically positioned within a clade encompassing Ixodidae-borne viruses associated with iguanas, small ruminants, seabirds and penguins distributed across different geographical areas. CTQV positioned differently depending on the segment considered, suggesting a possible reassortment at the origin of CTQV.

Conclusions: This study contributes to the knowledge of quaranjaviruses diversity in ixodid ticks from Eastern Europe. Further investigations are needed to assess virus infectivity and pathogenicity for vertebrates.

P 076

Molecular detection of *Borrelia* in *Ornithodoros Cavernicolous* collected in Araguapaz-Go, Brazil

M. Garcia Polli[1]*, M. M. Martins*[2]*, V. da Silva Rodrigues*[2]*, A. Suzin*[2]*, L. Miguel Rezende*[2]
R. da Costa Maia[2]*, S. Muñoz-Leal*[3]*, M. Pablo Juan Szabó*[2]*, J. Yokosawa*[1]
[1]Universidade Federal de Uberlândia, Microbiology, Uberlândia, Brazil
[2]Universidade Federal de Uberlândia, Faculdade de Medicina Veterinária, Uberlândia, Brazil
[3]Universidad de Concepción, Departamento de Ciência Animal, Chillán, Chile

Bacteria of the genus *Borrelia* are transmitted by ticks and may cause diseases to humans and other vertebrate animals; some of them may even be fatal and, therefore, have medical and veterinary importance. The present study aimed to obtain more information about the circulation of *Borrelia* spp. in Brazil. For this purpose, we searched for the DNA of these bacteria in *Ornithodoros cavernicolous* ticks from a bat rock shelter on a farm in the municipality of Araguapaz, in Goiás state, midwestern region of the country. DNA samples from 18 *O. cavernicolous* ticks were subjected to a two-round polymerase chain reaction (PCR) targeting a segment of the *Borrelia flaB* gene. Of the 18 tested, one sample amplified bacteria DNA, an infection rate of 5.6%. The nucleotide sequence of the amplified DNA was analyzed by Basic Local Alignment Search Tool Nucleotide (BLASTN) and showed 97.6% (290/297) identity with a DNA sequence of *Borrelia* detected in blood collected from a bat from Macaregua cave, in Colombia. Their deduced amino acid sequences were identical. A phylogenetic analysis showed that the sequence of the detected *Borrelia* sp. was most closely related with the *Borrelia burgdorferi* sensu lato group, with the sequence of *Borrelia afzelii* as the closest one, with 88.6% (263/297) nucleotide and 87.9% (87/99) amino acid identities. Further investigations should be carried out in order to determine whether the sequence we found belongs to a new taxon, as proposed for the *Borrelia* sp. of the Colombian study (Muñoz-Leal et al., 2021. DOI: 10.1111/zph.12789). *Ornithodoros carvernicolous* ticks are associated with bats and not likely to feed on humans. The *Borrelia* sp. described in this study may represent a new haplotype also associated with bats.

P 077

Global distribution and climate adaptation of *Ixodes trianguliceps*

F. Rubel[1]*, O. Kahl*[2]
[1]University of Veterinary Medicine Vienna, Vienna, Austria
[2]tick-radar GmbH, Berlin, Germany

The Palaearctic tick *Ixodes trianguliceps* Birula lives in the nests and burrows of its small mammal hosts (mainly *Myodes glareolus*, *Arvicola* spp., *Microtus* spp., *Apodemus* spp., *Sorex* spp.) and is – along with larvae and nymphs of *Ixodes ricinus* or *Ixodes persulcatus* – the most commonly collected tick species from these hosts in its Eurasian range. In this study, up-to-date maps depicting the geographical distribution and climate adaptation of *I. trianguliceps* are presented. A dataset was compiled, resulting in more than 1000 georeferenced locations. This data set covers the entire range of

I. trianguliceps for the first time. The distribution area between 8°W and 105°E and 40 – 69°N extends from Northern Spain to Western Siberia. It needs mentioning that endophilic ticks as soil organisms in contrast to non-endophilic ticks such as *I. ricinus* spend all off-host phases in a climatically rather protected environment and are much less exposed to extreme short-term weather conditions than the latter, especially when questing. To investigate the climate adaptation of *I. trianguliceps*, the georeferenced locations were superimposed on a high-resolution map of the Köppen-Geiger climate classification. The frequency distribution of the tick occurrence under different climates shows two peaks related to the following climates: warm temperate with precipitation all year round (Cfb), and boreal with warm or cold summers and precipitation all year round (Dfb, Dfc). Almost 97% of all considered *I. trianguliceps* locations are related to these climates. Thus, *I. trianguliceps* prefers climates with a warm or cold summer without dry periods. Cold winters apparently do not limit the distribution of this tick species, which has been recorded in the European Alps up to altitudes of 2300 m. A new detailed distribution map of *I. trianguliceps* in the greater area of the European Alps is shown. Conversely, *I. trianguliceps* does not occur in the Mediterranean area with its hot and dry summers.

P 078

Seroprevalence of *Rickettsia conorii* in dogs living in Northeastern Italy

S. Morelli[1], G. Simonato[2], M. Colombo[1], M. Grillini[2], A. Di Cesare[1], D. Traversa[1], R. Iorio[1]
A. Frangipane di Regalbono[2]
[1]University of Teramo, Department of Veterinary Medicine, Teramo, Italy
[2]University of Padua, Animal Health, Padua, Italy

Introduction: Tick-borne Mediterranean Spotted Fever (MSF) caused by *Rickettsia conorii* is a primary rickettsial disease of humans in Europe. Most canine infections by *R. conorii* are subclinical, despite a febrile illness can rarely occur. More importantly, dogs are epidemiological sentinels of MSF and act as a source of *R. conorii* for ticks and, indirectly, humans. Thus, monitoring the serological status of dogs living in endemic areas is pivotal under both Public Health and veterinary perspectives.

Objectives: This study has evaluated the seroexposure to *R. conorii* in dogs living in Northeastern Italy, to increase knowledge on the circulation of *R. conorii* in this geographic area, for which data available are scant.

Materials & Methods: A serum sample was obtained from 141 dogs, i.e. 37 and 104 in Veneto and Friuli-Venezia Giulia regions, with the consent of the owners in the framework of routine medical checks. All sera samples were subjected to a commercial IFAT (MegaFLUO RICKETTSIA conorii-Megacor Diagnostik GmbH) for the presence of antibodies against *R. conorii* according to manufacturer's indications.

Results: Overall, 74 dogs (52.5%), i.e. 19 (51.3%) and 55 (52.9%) in Veneto and Friuli-Venezia Giulia regions respectively were seropositive for *R. conorii*.

Conclusions: These rates suggest that dogs living in Northeastern Italy are at risk to be exposed to *R. conorii*. Despite *R. conorii* is one of the most widespread rickettsial pathogens affecting dogs, cross reactions with other *Rickettsia* species cannot be excluded. Regardless, the high level of exposure to *Rickettsia* spp. herein reported suggests that infected ticks are abundant in Northeastern Italy and that people living in this area are at potential risk of MSF. Constant surveillance, implementation of diagnostic tools and increased awareness towards rickettsial infections and other vector-borne pathogens are of utmost importance to safeguard health and welfare of dogs and humans.

P 079

Membrane feeding as a tool for production of “apo-symbiotic” *Ixodes ricinus* ticks

T. Hatalová[1,2], *M. Garcia Guizzo*[3,1], *J. Týč*[1], *M. Vancová*[1], *L. Žůrek*[3], *P. Kopáček*[1], *J. Perner*[1]
[1]Biology Centre of the Czech Academy of Sciences, Parasitology, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Faculty of Sciences, Ceske Budejovice, Czech Republic
[3]University of Veterinary and Pharmaceutical Sciences, Central European Institute of Technology (CEITEC), Centre for Zoonoses, Brno, Czech Republic

Introduction: Ticks are obligatory ectoparasites that transmit a large number of human and animal pathogens. Apart from pathogens, ticks harbour also other bacteria of possibly mutualistic relationship. The role of this bacterial microbiota as integral entity of ticks is still not completely understood. The tick *Ixodes ricinus* is stably occupied over generations by the bacterium *Midichloria mitochondrii*. This bacterium is mainly present in ovaries of fully engorged tick females, from where it is vertically transmitted into offspring.

Objectives: To elucidate the function of such a symbiosis, a so-called “apo-symbiotic” strain of *I. ricinus* is required. Various techniques have been described in the literature to generate apo-symbiotic ticks with various degrees of success. To address the lack of experimental opportunities, we designed and validated a novel approach, utilising a tick membrane feeding system, to produce apo-symbiotic *I. ricinus* ticks.

Materials & methods: Unlike microinjection of antibiotics, tick membrane feeding was efficient in clearing *M. mitochondrii* when ticks were fed the bovine blood supplemented with tetracycline. The level of *M. mitochondrii* was monitored with RT-PCR. To overcome off-target effects of tetracycline, we screened other antibiotics supplementation in the blood meal. Using Array Tomography, we analysed the structural integration of *M. mitochondrii* within mitochondria of tick ovarian cells.

Results: Depletion of *M. mitochondrii* in the apo-symbiotic *I. ricinus* ticks leads to a viable strain sustainable over multiple generations. The apo-symbiotic *I. ricinus* larvae, however, feed more slowly and less efficiently compared to controls.

Conclusion: Our results determine that membrane feeding system is an efficient tool for production of apo-symbiotic *I. ricinus* ticks.

References: DOI: 10.3389/fcimb.2022.1081666

P 080

Elusive *Borrelia* circulating in soft ticks infesting coastal birds in Portugal

A. C. Norte[1], *M. S. Núncio*[2], *J. M. Pereira*[1], *M. I. Laranjeiro*[1], *S. N. Veríssimo*[1], *C. Lopes*[1], *F. R. Ceia*[1] *V. H. Paiva*[1], *J. A. Ramos*[1], *I. Lopes de Carvalho*[2]
[1]MARE – Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[2]National Institute of Heatlh Doutor Ricardo Jorge, Centre for Vector and Infectious Diseases Research, Setúbal, Portugal

Borrelia burgdorferi s.l. circulates in terrestrial and also in marine cycles involving *Ixodes uriae* and, mainly, *Borrelia garinii*.
During the annual fieldwork campaigns to monitor coastal and pelagic birds' breeding colonies in 2019, we collected 4 larval tick specimens infesting two yellow-legged gull (*Larus michahellis*) chicks from Berlenga Island and Peniche, Portugal. These ticks were molecularly identified as *Alectorobius capensis* through 16S sequencing, which represented the first detection of this tick species in Portugal. Moreover, these ticks were tested for *B. burgdorferi* s.l. infection, and one specimen was positive by real time PCR targeting the 23S region. To identify the genospecies, a nested PCR targeting the *flaB* gene was performed followed by sequencing. This allowed the identification as *Borrelia turdi*. Between 2019 and 2022, blood samples were collected from adult yellow-legged gulls ($n = 43$) at Berlenga Island, Peniche and Porto, Audouin's gull *Ichthyaetus audouini* ($n = 14$) at Ilha Deserta, Algarve and Cory's shearwater *Calonectris borealis* ($n=8$) at Berlenga island in order to screen serum samples for anti-*Borrelia* IgG antibodies through a highly sensitive commercial ELISA containing whole cell extracts of *B. afzelii*, *B. garinii*, and *B. burgdorferi* as well as recombinant OspC and VlsE. However, we did not detect antibodies in any of the seabird samples. These samples will be further tested by IFA using *B. burgdorferi* s.l as antigen to increase the spectrum of *Borrelia* antibodies detected. Further studies are needed to better understand tick infestations and *Borrelia* cycles occurring in these biological communities of pelagic and coastal seabird species, and in both adults and chicks.

P 081

Microbial composition in *Hyalomma anatolicum* collected from livestock in the United Arab Emirates using next-generation sequencing

N. Perveen[1], *S. B. Muzaffar*[1], *R. Vijayan*[1], *M. A. Al-Deeb*[1]
[1]United Arab Emirates University, Biology, Al Ain, United Arab Emirates

Introduction: *Hyalomma anatolicum* is a widely distributed tick species that acts as a vector transmitting tick-borne pathogens (TBPs) in livestock. Such pathogens affect the health of livestock and consequently reduce their productivity. Knowledge about the microbial communities (pathogens and endosymbionts) of ticks in the United Arab Emirates (UAE) is scarce.

Objectives: The aim of the present study was to quantify microbial diversity in *H. anatolicum* using next-generation sequencing (NGS) technology.
Methods: *Hyalomma anatolicum* ticks were collected from livestock in the emirates of Abu Dhabi, Dubai, and Sharjah in the UAE in 2019. DNA was extracted from 175 male ticks sampled from livestock (n = 78) and subjected to NGS. The 16S rRNA gene was analyzed using the Illumina MiSeq platform to determine the bacterial communities. Principal coordinates analysis (PCA) was performed to identify patterns of diversity in the bacterial communities.
Results: Twenty six bacterial families with high relative abundance were identified, of which the most common were Staphylococcaceae, Francisellaceae, Corynebacteriaceae, Enterobacteriaceae, Moraxellaceae, Bacillaceae, Halomonadaceae, Xanthomonadaceae, Pseudomonadaceae, Enterococcaceae, Actinomycetaceae and Streptococcaceae. The diversity of the microbial communities in terms of richness and evenness was different at the three study locations, with the PCA showing clear clusters separating the microbial communities in ticks collected at Abu Dhabi, Dubai, and Sharjah. The presence of bacterial families harboring pathogenic genera showed that *H. anatolicum* could pose a potential threat to livestock and food security in the UAE.
Conclusions: The study is the first to document important data on the microbial communities associated with *H. anatolicum* in the UAE. This knowledge will facilitate a better understanding of the distribution pattern of microbes in livestock ticks in the UAE.

P 082

First molecular detection of multiple vector borne diseases in pet dogs and cats of Hongkong

S. Hussain[1], *M. U. Aziz*[1], *A. Hussaain*[2], *B. Song*[1], *B. Song*[1], *J. Zeb*[1], *O. A. Sparagano*[1]
[1]City University of Hong Kong, Hong Kong, Hong Kong
[2]University of Illinois, Chicago, IL, United States

In recent years, the incidence of vector-borne diseases has increased throughout the globe. In particular, *Ehrlichia canis*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi* and *Babesia gibsoni* and *Dirofilaria immitis* diseases pose a burden on animal health. Nevertheless, there have been no studies undertaken on the occurrence of VBDs in pet dogs and cats in Hong Kong SAR. This study fills this gap, and is the first to determine the seroprevalence of major VBDs, such as *D. immitis*, *E. canis*, *E. ewingii*, *A. phagocytophilum*, *A. platys* and *B. burgdorferi* s.s., in dogs and cats through commercially available SNAP 4Dx plus testing. Infection by all these pathogens and *Babesia* sp. was further assessed through PCR and DNA sequencing. A total of 224 blood samples were collected from domestic dogs (n = 159) and cats (n = 65) in Hong Kong SAR during summer 2022. Hematocrit and platelet counts were determined in each blood sample and other hematological parameters were assessed using an automatic hematology analyzer. All cat sera samples were negative for tested pathogens, but antibodies against some of the pathogens were detected in dog sera samples. Here, the highest figures were recorded for seroprevalence of *E. canis*/*E. ewingii* (10.69%), followed by *D. immitis* (5.67%), and *A. phagocytophilum*/*A. platys* (2.51%). No *B. burgdorferi* s.s. antibodies were detected in any of the dogs tested. Through molecular diagnostics, we detected the presence of *B. gibsoni* (3.77%), *E. canis* (3.14%), *D. immitis* (5.67%), and *A. phagocytophilum* (1.26%). Neighbor-Joining phylogenetic trees for vector-borne pathogens (i.e., *A. phagocytophilum*) showed 100% clustering to Japan, the USA and Germany, whereas *E. canis* showed 100% clustering to China, Turkey, Cuba, and Greece. Similarly, *B. gibsoni* clustered 100% to India, Sri Lanka and Austria, while *D. immitis* clustered in Iraq, South Korea, Portugal, France, the USA and Italy. This study provides the first evidence on the occurrence of tick-borne pathogens in pet dogs in Hong Kong SAR.

P 083

One health approach in research, diagnostic and xenodiagnostic of tick-borne diseases – holistic model from Serbia

P. Banović[1]*, D. Mijatović*[1]*, V. Simin*[1]*, I. Bogdan*[1]*, A. A. Díaz-Sánchez*[2]*, A. Wu-Chuang*[3]*, A. Maitre*[3]
D. Obregón[4]*, S. Moutailler*[3]*, A. Cabezas-Cruz*[3]
[1] Pasteur Institute, Novi Sad, Serbia
[2]University of Saskatchewan, Saskatchewan, Canada
[3] ANSES, INRAE, Ecole Nationale Vétérinaire d’Alfort, UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, France
[4]University of Guelph, School of Environmental Sciences, Ontario, Canada

Objectives: To propose a “One Health” interdisciplinary approach to study the spread of tick-borne pathogens (TBPs) between ticks, animals and humans, as well as integration of xenodiagnostic procedures in healthcare management of humans with tick infestation.

Subjects and Methods: Throughout 4 consecutive years (2019–2022) we conducted a series of studies under “One Health” approach which included enrollment of patients and ticks removed from them, as well as investigation of possible TBP foci within household of patients with emerging TBD (1–5). Materials and datasets generated through this approach were analyzed via entomologic, microbiological and molecular analysis, including microfluidic real-time high-throughput PCR system that was used to test the DNA of the tick and human blood for the presence of 27 bacterial and eight parasitic TBPs.

Results: We identified *Ixodes ricinus* as most frequent tick species infesting humans. Different *Rickettsia* species were the most common TBPs identified. *Rickettsia helvetica* infection was identified to have significant impact on tick microbiota, whereas some taxa were identified as to facilitating *R. helvetica* infection in ticks. During the years 2020 and 2021 we detected and described cases of atypical infections caused by *R. helvetica* and *R. raoultii*, as well as the case of hypersensitivity reaction after a tick bite. In addition, we examined the platelet fraction from patient blood as a substrate for direct detection of TBPs.

Conclusions: The One Health approach described in Serbia is the first implemented worldwide and will help to characterize the components of the chain of infection leading to human infection by TBPs as well as diagnostics of tick-borne diseases caused by emerging pathogens.

P 084

Affinity-based proteomic mapping of lipid hydrolases in the midgut of hard tick *Ixodes ricinus*

T. Kozelková[1,2], *P. Kopáček*[2], *M. Schittmayer-Schantl*[3], *R. Birner-Gruenberger*[3]
[1]Faculty of Science, Department of Chemistry, Ceske Budejovice, Czech Republic
[2]Biology Centre of the Czech Academy of Sciences, Parasitology, Ceske Budejovice, Czech Republic
[3]Vienna University of Technology, Institute of Chemical Technologies and Analytics, Vienna, Austria

Introduction: Physiology, development, reproduction, and vectorial capacity of ticks depend entirely on the ingestion and processing of host blood as the sole source of nutrients. In contrast to the relatively well-studied digestion of proteins, almost nothing is known about the uptake and utilization of host lipids. Several genes encoding lipolytic enzymes (lipases and hydrolases) have been identified in the transcriptomes of the midgut of our model tick, *Ixodes ricinus*. However, identification of the corresponding proteins by classical proteomic approaches is hindered by the presence of other highly abundant proteins.
Objectives: To overcome this obstacle, we used the activity-based proteomics approach (ABP) to identify lipid hydrolases in the proteome of the midgut of *I. ricinus*.
Materials and methods: Midgut homogenates from different feeding time points (unfed, fed for 3 and 5 days, and fully fed) were labeled with C6 ABP and active hydrolases were isolated using streptavidin-agarose beads. On-bead digestion using trypsin was followed by the peptide analysis carried out on the timsTOF Pro (Bruker). Label-free quantitative proteomics (LFQ) was used to investigate the dynamic changes during the tick blood meal. Data were analyzed using Perseus and MaxQuant programs.
Results: Several hydrolases from different classes were identified during the adult female blood meal. LFQ values showed different abundance of some hydrolases at different time points of feeding. The obtained results were validated by qRT-PCR expression analysis of the corresponding mRNAs.
Conclusion: Our first-ever activity-based proteomics study of lipid hydrolases contributes to the limited understanding of lipid metabolism in ticks and holds promise for finding susceptible targets for effective interventions against ticks.

Supported by GACR 21–08826S and ERDFunds
(No.CZ.02.1.01/0.0/0.0/16_019/0000759)

P 085

Passive surveillance on exotic tick species in Sweden – results from 2019–2022

G. Grandi[1,2], *L. Chitimia-Dobler*[3], *A. Omazic*[4], *P. Choklikitumnuey*[1,2], *A. Albihn*[2,5], *D. Perissinotto*[1]
[1]National Veterinary Institute (SVA), Microbiology, Uppsala, Sweden
[2] Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Publich Health (BVF), Uppsala, Sweden
[3]Bundeswehr Institute of Microbiology, Munich, Germany
[4]National Veterinary Institute (SVA), Department of Chemistry, Environment, and Feed Hygiene, Uppsala, Sweden
[5]National Veterinary Institute (SVA), Department of Disease Control and Epidemiology, Uppsala, Sweden

Introduction: Repeated findings of adults of alien tick species (i.e., *Hyalomma marginatum*, *H. rufipes* and *Dermacentor reticulatus*) occurred in Sweden in the past 10 years. Climate change can explain this phenomenon. Alien ticks' monitoring is relevant since they could introduce new zoonotic tick-borne pathogens.

Objectives: To involve the public into reporting the occurrence of alien ticks in Sweden and by doing this i) register alien ticks' findings and getting the specimens sent to the National Veterinary Institute (SVA, Uppsala) when available and ii) increase preparedness against new tick-borne diseases in Sweden.

Materials & methods: In 2019 SVA started encouraging the public to send pictures of uncommon ticks. To avoid unappropriated reports, photos of *Hyalomma* spp. and *D. reticulatus* were provided onto the webpage dedicated to alien tick collection (https://www.sva.se/amnesomraden/smittlage/insamlingar/insamling-av-ovanliga-exotiska-fastingar/). Whenever the photo sent was considered belonging to an alien tick species, it was asked to send the tick(s) to SVA. Ticks received were identified to the species level by morphological keys and stored at -80˚C waiting for molecular and microbiological analysis.

Results: The majority of photos sent were from *Ixodes* spp. ticks. Photos of less common tick species were also sent, i.e., *Ixodes hexagonus*, *Haemaphysalis punctata,* and *Carios vespertilionis*. Regarding alien tick species, SVA received photos of adult *Hyalomma* spp. (n=2 in 2019, n=4 in 2020, n=3 in 2021, and n=2 in 2022, see a picture example, Figure 1) and adults and nymphs of *Dermacentor reticulatus* (n=2 in 2019, n=1 in 2020, n=1 in 2021, and n=2 in 2022, see a picture example, Figure 2).

Conclusion: Thanks to public's contribution it has been possible i) to confirm the possibility of *Hyalomma* nymphs to develop to the adult stage in Sweden and ii) at least two established endemic foci of *D. reticulatus* have been identified for the first time in Sweden.

P 086

A systematic review on the prevalence of tick-borne encephalitis infection in milk and milk products in Europe

L. Tomassone[1], J. Leonardi-Bee[2], A. Mannelli[1], A. Vicentini[1], E. Martello[2]
[1]University of Turin, Department of Veterinary Sciences, Turin, Italy
[2]University of Nottingham, Division of Epidemiology and Public Health, School of Medicine, Nottingham, United Kingdom

Introduction: Tick-borne encephalitis is a vector-borne disease caused by a Flavivirus, the tick-borne encephalitis virus (TBEV). Although TBEV is mainly transmitted to humans by tick bite, the alimentary route is also possible.

Objectives: This systematic review (SR) was conducted to assess the prevalence of TBEV in milk and milk products from domestic ruminants in Europe.

Materials & methods: A comprehensive search was performed in three databases (Medline, Embase and CAB Abstracts, from 1980 to November 2022). Screening, data extraction and critical appraisal were conducted independently by two reviewers. TBEV prevalence was calculated using the number of milk or milk product samples tested (RNA or specific anti-TBEV antibodies), and number of positive samples. A narrative synthesis was performed.

Results: 396 articles were identified from the searches, 58 were selected for full-text screening, and 15 were included. Data from 32 data collections were extracted (27 on milk and 5 on cheese), of which the sample size ranged from a single sample (9 studies) to 1363 samples in one study. Regarding animal species, data collections focused on cow milk ($n=14$), goat milk ($n=10$), sheep milk ($n=2$), goat cheese ($n=4$), cow and goat cheese ($n=1$) and sheep and goat milk ($n=1$). Fifteen studies on milk were carried out on individual animal samples and 12 on bulk-tank milk. Studies with larger milk samples ($n\geq29$; corresponding to the sample size needed to detect at least one positive sample, with an expected prevalence of 10%, and 95% confidence level) had a median prevalence of infection of 4.5% (range 0% to 20.7%). Overall, 10.1% of cheese specimens were positive (all studies combined).

Conclusion: Our SR confirmed that unpasteurised milk or milk products from domestic ruminants can be a vehicle of TBEV transmission to humans. Therefore, surveillance on these products could be a valuable tool for studying the epidemiological situation in a geographic area.

P 087

Molecular screening of *Borrelia miyamotoi* and Tick-Borne Encephalitis Virus in ixodid ticks in urban green areas in Serbia

G. Veinovic[1]*, D. Mihaljica*[1]*, R. Sukara*[1]*, A. Vasic*[2]*, I. Djuric Maslovara*[3]*, O. Vukicevic Radic*[3]
M. Rajkovic[3]*, R. Zlatic Sibinovic*[3]*, S. Tomanovic*[1]
[1]University of Belgrade, Institute for Medical Research, National Institute of Republic of Serbia, Group for Medical Entomology, Centre of Excellence for Food and Vector-Borne Zoonoses, Belgrade, Serbia
[2]Scientific Veterinary Institute of Serbia, Department for Bacteriology and Parasitology, Belgrade, Serbia
[3]Public Utility Companies "City Sanitation", Sector of Ecology and Environmental Improvement, Belgrade, Serbia

Introduction: Zoonotic tick-borne diseases (TBDs) represent an increasing threat in urban areas, including city parks and green recreational areas. *Borrelia miyamotoi* is pathogenic to humans but there is still little information about its circulation in nature and potential local impact on human health, while tick-borne encephalitis virus (TBEV) is endemic in many European countries and sporadic autochthonous cases in humans have been reported in Serbia.

The objective of our study was to determine the prevalence of two emerging zoonotic tick-borne pathogens (TBPs) – *B. miyamotoi* and TBEV in urban areas in the city of Belgrade (24 localities), four localities in Eastern Serbia, one in the province of the Vojvodina.

Methods: At 29 localities, a total of 480 ticks were collected from March to June 2021, and identified to the species level by using morphological keys. DNA and RNA were extracted from individual tick samples, while for molecular detection, probe-based qPCR and nested PCR were applied.

Results: Among 480 ticks, 445 were identified as *Ixodes ricinus* (213 males, 176 females, 56 nymphs), 30 as *Dermacentor reticulatus* (18 females, 12 males), three *Dermacentor marginatus* females, one *Haemaphysalis concinna* female, and one *Haemaphysalis punctata* male. Using probe-based qPCR, the prevalence of *B. miyamotoi* in ticks was 1.04% (DNA was detected in five *I. ricinus*, two females and two males collected from three Belgrade localities and one male from Eastern Serbia), while sequencing was successful in samples of two *I. ricinus* females collected from two Belgrade localities and one male from Eastern Serbia. TBEV RNA was not detected in any sample.

Conclusions: This is the first report on the presence of the emerging zoonotic pathogen *B. miyamotoi* in *I. ricinus* ticks in urban green areas in Serbia, indicating the risk of *B. miyamotoi* disease. Risk areas within cities should be identified and knowledge regarding TBPs and TBDs among the general population in urban areas should be increased.

P 088

Experimental infection with a novel *Babesia* sp. – Evaluation of pathogenicity in healthy and immunosuppressed goats

M. C. Ulucesme[1], *S. Ozubek*[1], *M. Aktas*[1]
[1]Firat University, Faculty of Veterinary Medicine Department of Parasitology, Elazig, Turkey

Small ruminant babesiosis is a common tick-borne disease in Turkiye. Recently, a novel *Babesia* sp. infecting goats was detected in the Mediterranean region of Turkiye, and isolated from a naturally infected goat. An experimental study was conducted, and the pathogenicity of this novel *Babesia* species was investigated. For this purpose, experimental groups were formed from 4–8-month-old spleen-intact (n=6) and immunosuppressed goats (n=7). The *Babesia* sp. stabilate with 10% parasitemia was injected 15 ml into the immunosuppressed group and 30 ml into the spleen-intact group. After both groups were infected, all went under routine examinations and the course of infection was tracked. The first piroplasm forms were detected in peripheral blood 3–7 days after inoculation in the immunosuppressed group, and they all developed severe clinical signs of babesiosis such as high fever, hemoglobulinuria and anemia. The parasitemia was ranging from 6.5 to 41.8%, and only 2 out of 7 immunosuppressed goats survived the disease. In the spleen-intact group, only 4 out of 6 goats had clinical symptoms. Babesiosis clinical symptoms except for high fever were not observed but symptoms like loss of appetite and depression lasted 2–3 days in this group. The first piroplasm forms were detected 2–5 days after inoculation, and parasitemia lasted 3–6 days in the spleen-intact group. In the immunosuppressed group, RBC, WBC, HCT, HB and PLT levels were lower than before and also biochemistry parameters showed differences in glucose, ALT and AST values. There was no significant difference in hematological parameters in the spleen-intact group and only a decrease in CK value was observed. As a result, *Babesia* sp. caused a highly pathogenic and severe clinical infection in immunosuppressed goats while causing a disease with mild clinical findings in healthy individuals. This work was supported by the Scientific and Technological Research Council of Turkiye (TUBITAK, project no. 1180871).

P 089

Citizen science tick monitoring program to reveal emergence of *Hyalomma* ticks and potential pathogens in Hungary

É. Szabó[1,2], *G. E. Tóth*[3,4], *Z. Tauber*[3,4], *G. Kemenesi*[3,4], *G. Földvári*[1,2]
[1]Centre for Ecological Research, Institute of Evolution, Budapest, Hungary
[2]Centre for Eco-Epidemiology, National Laboratory for Health Security, Budapest, Hungary
[3]University of Pécs, National Laboratory of Virology, Pécs, Hungary
[4]University of Pécs, Faculty of Sciences, Pécs, Hungary

Hyalomma ticks are important vectors of Crimean-Congo haemorrhagic fever virus (CCHFV) and other pathogens. They are frequently carried as immatures from Africa and Mediterranean areas to temperate Europe by migratory birds and emergence of adults has been reported in many countries so far considered non-endemic. This study aimed to implement the first steps of the DAMA (Document, Assess, Monitor, Act) protocol by monitoring the potential arrival of adult *Hyalomma* ticks in Hungary applying citizen-science methods. The first year of the program www.kullancsfigyelo.hu had a strong start, the website designed to inform the public exceeded 31,000 visitors in 7 months, and we received hundreds of photos and observations from the participants. Among them, we successfully identified one male *Hyalomma marginatum* and one male *Hyalomma rufipes*. Sequencing confirmed the identification for both ticks. None of the specimens carried CCHFV. Based on the first year's promising results, the research continued in 2022. This year, among other indigenous tick species we identified 9 ticks as *Hyalomma* from around the same number of submissions as in the previous year. In addition, based on the geographical distribution of the 9 specimens, two aggregation points could be observed, where more than one tick was found. We also summarized the scattered historical reports about the occurrence of *Hyalomma* ticks and CCHFV in Hungary. An adult specimen of *H. marginatum* was subjected to Illumina-based viral metagenomic sequencing. We identified sequences of the so far uncharacterized *Volzhskoe tick virus*, an unclassified member of the *Bunyavirales* order. The virus was first discovered in a *H. marginatum* tick in 2018, in western Russia. Here we present the genomic and phylogenetic characterization of the same virus from Hungary. Our work demonstrates how citizen science engagement can contribute to tick and virus monitoring, discovery and thus preventive measures.

P 090

Comparative blocking efficacy of two ectoparasiticides against the acquisition of *Borrelia burgdorferi* by *Ixodes ricinus* and *Ixodes scapularis* ticks in a canine ex vivo model

D. Tahir[1], *S. Dupuis*[2], *V. Geolier*[2], *N. Lekouch*[3], *V. Choumet*[1], *M. Varloud*[2]
[1] Pasteur Institute, Paris, France
[2]Ceva Santé Animale, Libourne, France
[3]Clinvet Morocco, Mohammedia, Morocco

Introduction: protecting dogs from tick infestation is the first line of protection against tick-borne diseases like Lyme borreliosis. Many pharmaceutical compounds are available against ticks, but they differ widely in term of protection features. The objective of this study was to compare the blocking efficacy of two ectoparasiticides against the acquisition of *Borrelia burgdorferi* by *Ixodes ricinus* and *I. scapularis* ticks using an ex vivo model.

Methods: thirty-two dogs were assigned to three groups: fluralaner [F, Bravecto®] treatment group (n= 8) administered as a single oral treatment on day 0, dinotefuran-permethrin-pyriproxyfen [DPP, Vectra®3D] treatment group (n= 8) topically treated on day 56, and non-treated control group (n= 16). Hair and blood samples were collected from each dog on days 57, 62, 69, 76 and 83. Hair was added to the membrane separating two glass chambers of feeding units (FU). One chamber was filled with blood previously spiked with *B. burgdorferi* (105 cells/ml). The second chamber was filled with 20 adult ticks of *I. ricinus* or *I. scapularis*. The FU were monitored from 1 h up to 72 h after seeding during which blood was changed, tick attachment and presence of feces was assessed. The uptake of *B. burgdorferi* was determined in ticks using nested PCR.

Results: in the non-treated control group, tick feces and positive *Borrelia* ticks were observed at each time point. In the DPP-treated group, whatever the tick species, ticks (100%) were found dead within 1 h after seeding. All of the randomly selected female ticks from the DPP-treated group tested negative for *B. burgdorferi*. In the F-treated group, the acaricidal efficacy reported at 12 h was <25% and <20% for *I. ricinus* and *I. scapularis*, respectively. In addition, several female ticks tested positive for *B. burgdorferi* DNA.

Conclusion: in this study, the protective effect of DPP against the acquisition of *B. burgdorferi* was confirmed. Furthermore, our results demonstrated the superiority of DPP compared to F. in the speed of killing activity against ticks as well as in preventing the acquisition of *B. burgdorferi* by *I. ricinus* and *I. scapularis*.

P 091

Resources for ticks and tick pathogens at the eukaryotic pathogen, vector and host informatics resources (VEuPathDB.org)

S. Kelly[1]
[1]VEuPathDB/Imperial College London, Life Sciences, London, United Kingdom

VectorBase (VectorBase.org) and PiroplasmaDB (PiroplasmaDB.org) are part of the VEuPathDB project, a NIAID-funded bioinformatics resource center (BRC) that provides free, online access to genomic-scale data-mining resources for >500 species of eukaryotic pathogens and related taxa, including protozoan parasites, fungi and oomycetes, arthropod vectors of disease, and selected host species. VEuPathDB resources empower end-users to leverage diverse multi-Omics datasets, without requiring specialised analytical or computational skills. Our internal pipelines analyse a wide variety of omics data and couple the analysis results to data mining capabilities, data visualisations, and custom tools to facilitate the discovery of meaningful relationships from large volumes of data. VectorBase and PiroplasmaDB have 55 and 18 genomes respectively, including *Ixodes*, *Babesia*, and *Theileria* species; new genomes are made available on a regular basis. Importantly, the search strategy system allows the development of in silico experiments consisting of combining results from different experiments and analyses. The MapVEu tool, a data visualisation and exploration tool, integrates geospatial genomic, phenotypic and population data for traits such as population abundance, pathogen infection status, insecticide resistance genotypes and phenotypes, genetic variation with microsatellites, chromosomal inversions and SNPs and blood meal identification. Currently, MapVEu includes surveillance data, genetic variation data for ticks in the US and a number of pathogen infection studies. Raw data sets can be downloaded from the map interface. Our active user support offers an email help desk, social media, video tutorials, a webinar series, and a worldwide program of workshops. Please email us at help@VEuPathDB.org for more information.

P 092

Genetic characterization of a *Rickettsia parkeri* strain in endemic areas of the coast of São Paulo state, Brazil

M. P. D. Albuquerque[1,2], *G. A. C. Takeda*[1,2], *D. R. Xavier*[1,2], *C. Scinachi*[1,2], *N. O. D. Moura-Martiniano*[3] *A. Pinter*[1,2]
[1]University of São Paulo, Instituto Pasteur, São Paulo, Brazil
[2]University of São Paulo, Faculdade de Saúde Pública, São Paulo, Brazil
[3]Janssen-Cilag Farmaceutica, Immunology, São Paulo, Brazil

Spotted Fever is an acute febrile infectious disease, with varying severity. In some regions of the Americas, a milder form of the disease is caused by the species *Rickettsia parkeri*, which is characterized by milder clinical manifestations. Currently, there are few epidemiological studies on the disease and its etiological agent. This work aims to better understand the epidemiology of the disease by conducting a transversal phylogenetic survey in different strains of *R. parkeri* of ticks and their dispersion in different endemic areas of the State of São Paulo. Tick collections were made in dogs, identification of tick species, and subsequent molecular detection, phylogenetic analysis, a study of inter and intraspecific genetic diversity of *R. parkeri* and geospatial and landscape comparison between the different sampling sites. The results contribute to a better understanding of the genetic distribution and flow between different strains of *R. parkeri* existing in these regions and helps to elucidate their abundance and distribution, and consequently, contributing to the knowledge of the epidemiology of mild spotted fever, which is still incipient.

P 093

Detection of *Anaplasma* spp., *Ehrlichia* spp., and *Neoehrlichia* spp. in *Ixodes granulatus* from wild animals in Kinmen County, an offshore island of Taiwan

C. Y. Lin[1], *T. Y. Yen*[1], *Y. W. Wu*[2], *H. C. Wang*[1], *K. H. Tsai*[1]
[1]National Taiwan University, Institute of Environmental and Occupational Health Sciences, College of Public Health, Taipei, Taiwan
[2]National Quemoy University, Department of Food Science, Kinmen, Taiwan

Introduction: In Taiwan, Lyme disease and severe fever with thrombocytopenia syndrome (SFTS) are listed in the notifiable diseases by the Taiwan CDC. Reportable vector-borne diseases are infections transmitted by the bite of infected arthropod species, such as mosquitoes, ticks, lice and mite. Previous study has found *Ehrlichia chaffeensis* in *Rhipicephalus haemaphysaloides* and *Ixodes granulatus* ticks in rodents. Human cases of granulocytic anaplasmosis and ehrlichiosis have been demonstrated in Taiwan in recent years. However, systematic surveillance of *Ehrlichia* spp. and *Anaplasma* spp. from vectors in Taiwan are limited.
Objectives: Tick-borne disease are neglected in Taiwan. The study intend to identify vector ticks and tick-borne pathogens from wild animals. This data will provide basic information and delineate the relationship between tick-borne pathogens and fever of unknown origin on offshore island.

Materials & methods: Wild animals were captured by rodent trap with tomato baits. Ectoparasites of ticks and mites were collected from rodents. Their blood, spleen and liver tissues were all collected for following analysis. Nested-PCR with the primers of 16S and *p44/msp2* gene were used for the detection of *Ehrlichia* spp. and *Anaplasma* spp. The PCR products were sequenced in both forward and reverse directions. A phylogenetic tree was constructed based on the alignment and the most closely related paralogs, followed by the neighbor-joining method with 1,000 bootstrap re-samplings. Results: A total of 692 rodents were trapped from three region around Kinmen from August 2018 to June 2022. Two rodent species (*Rattus losea* and *Rattus tanezumi*), one Pallas's squirrel (*Callosciurus erythraeus*), and one insectivore species (*Suncus murinus*) were collected. Major tick species was *Ixodes graunlatus*. The positivity rate of *Anaplasma phagocytophilum*, *Candidatus* Neoehrlichia mikurensis, *Ehrlichia* spp., *Candidatus* Lariskella sp. in *I. graunlatus* ticks was 2.7% (1/37), 18.9% (6/37), 2.8 (1/37) and 2.8 (1/37), respectively. The sequences of these novel isolates revealed the diversity of *Ehrlichia* and *Anaplasma* other than *Rickettsia* spp. in Kinmen, an offshore island of Taiwan. Conclusion: This study showed diverse rickettsia, including *Anaplasma phagocytophilum*, *Candidatus* Neoehrlichia mikurensis, *Ehrlichia* spp., *Candidatus* Lariskella sp., carried by *Ixodes granulatus* for the first time. Whether they cause human diseases on offshore island remain to be studied.

P 094

Specificity of MurE and the role of ornithine in pathogenesis of the Lyme disease spirochete *B. burgdorferi*

B. Hart[1], *R. Helm*[1], *P. Sobrado*[1,2,3], *B. Jutras*[1,2,3,4,5,6]
[1]Virginia Tech, Biochemistry, Blacksburg, VA, United States
[2]Virginia Tech, Center for Drug Discovery, Blacksburg, VA, United States
[3]Virginia Tech, Fralin Life Sciences Institute, Blacksburg, VA, United States
[4]Virginia Tech, Molecular and Cellular Biology, Blacksburg, VA, United States
[5]Virginia Tech, Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Blacksburg, VA, United States
[6]Virginia Tech, Translational Biology, Medicine, and Health, Blacksburg, VA, United States

Lyme borreliosis (LB) is a tick-borne disease caused by the spirochete *Borrelia burgdorferi*. Current treatments for LB include an intense 14- to 21-day course of broad-range antibiotics that can result in nausea, diarrhea, and weight loss due to disruption of the gut microbiome. Even with current antibiotic treatments, ~20% of patients will develop chronic Lyme arthritis, the cause of which is unknown. *B. burgdorferi* lacks classical virulence factors, however, is known to shed unique peptidoglycan (PG) fragments, which are recognized by the host immune system and have been linked to LB pathogenesis. PG is an essential component of bacteria and is made of repeating sugars cross-linked by peptides. Gram-positive bacteria typically contain L-lysine at the third position of the peptide chain, whereas Gram-negative bacteria typically contain meso-diaminopimelic acid (mDAP). Interestingly, as an atypical Gram-negative bacterium, *B. burgdorferi* contains an ornithine in this position. The enzyme that incorporates this amino acid is MurE. Our objectives are to biochemically characterize MurE of *B. burgdorferi*, as well as elucidate the role of ornithine in PG for the pathogenesis of *B. burgdorferi*. Using computational tools, investigation into the MurE sequences has revealed a unique motif in *B. burgdorferi* MurE that provides evidence for an affinity towards ornithine over lysine and mDAP. To further explore this, a series of molecular and cellular studies were utilized to begin to define mechanistic details that underlie substrate specificity. Using cutting-edge heterologous replacement studies in live bacteria, we are not only defining the role of ornithine in PG pathogenesis, but the physiological consequences of this atypical alteration. These studies will provide a deeper understanding of an essential enzyme for all bacteria that can be utilized to develop novel therapies for LB, as well as develop a tool that can be used to explore fundamental concepts in immunobiology.

P 095

Efficacy and safety of pharmacological treatments for Lyme neuroborreliosis – Update of a systematic review

R. Dersch[1], *S. Rauer*[1]
[1]University of Freiburg, Clinic of Neurology and Neurophysiology, Medical Center – University of Freiburg, Faculty of Medicine, Freiburg i.Br., Germany

Background: For supporting the development of the German guideline on Lyme neuroborreliosis (LNB), a systematic review on pharmacological treatments of Lyme neuroborreliosis was conducted in 2015. Since then, several newer studies regarding treatment of LNB were published. To incorporate the available literature, an update of the systematic review was perfomed. Methods: A systematic literature search on Medline and CENTRAL was performed using a pre-specified search strategy including studies published from 2014–2022. Randomized controlled trials (RCTs) and non-randomized studies (NRS) were evaluated. Risk of Bias was assessed using the Cochrane risk of bias tool and the ROBINS-I-tool for non-randomized studies. The primary outcome was “residual neurological symptoms”. The quality of the evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. Results: After screening 911 records, 2 RCTs and 5 NRS were additionally identified. Risk of bias in NRS was generally high. Data from one RCT could be integrated in an existing meta-analysis, showing no statistically significant difference between doxycycline and beta-lactam antibiotics regarding residual neurological symptoms at 12 months (RR 0.98, 95% CI 0.76–1.26) or adverse events (RR 0.9, 95% CI 0.63–1.39). GRADE-Assessment of the body of evidence regarding these outcomes has improved to “moderate” compared to the previous review. Three NRS show no benefit for additional steroid use in LNB with facial palsy, however risk of bias is critical in these studies. Conclusions: New RCTs add to the certainty of previous analysis showing no statistically significant difference between treatment with doxycycline and beta-lactam antibiotics in LNB. NRS do not suggest a role for steroids in facial palsy due to LNB, however, certainty is reduced due to considerable risk of bias.

P 096

R. sanguineus from Hungarian dogs – identification and detection of tick-borne pathogens

S. Ghodrati[1,2], *P. M. Lesiczka*[3], *L. Žůrek*[4,5,6], *F. Szekely*[7], *D. Modrý*[1,2,3]
[1]Masaryk University, Department of Botany and Zoology, Faculty of Science, Brno, Czech Republic
[2]Biology Centre of the Czech Academy of Sciences, Biology Centre, Institute of Parasitology, České Budějovice, Czech Republic
[3]Czech University of Life Sciences Prague, Department of Veterinary Sciences/CINeZ, Faculty of Agrobiology, Food and Natural Resources, Prague, Czech Republic
[4]University of Veterinary Sciences, CEITEC, Brno, Czech Republic
[5]Mendel University, Department of Chemistry and Biochemistry, Brno, Czech Republic
[6]Czech University of Life Sciences Prague, Department of Microbiology, Nutrition and Dietetics/CINeZ, Faculty of Agrobiology, Food and Natural Resources, Prague, Czech Republic
[7]1095 Budapest Lechner Ödön Fasor 10/B, Budapest, Hungary

Introduction: The brown dog tick, *Rhipicephalus sanguineus* sensu lato (s.l.) is a part of a complex of species with an unsettled species concept. A geographic pattern in the distribution of main lineages of ticks included in *R. sanguineus* s.l. was repeatedly discussed and major clades were commonly termed as "temperate lineage" (nowadays *R. sanguineus* s.str.) and a "tropical lineage" (nowadays *R. linnaei*). In Europe, *R. sanguineus* has been considered mainly a Mediterranean tick species with sporadic findings in northern countries (e.g. U.K., Ireland, Denmark, and Sweden). Due to global climatic changes, northward expansion of this thermophilic tick distribution range is observed, and it was reported as far north as Hungary. In Europe, *R. sanguineus* is known as a vector of a range of pathogens of medical and veterinary importance, including *Anaplasma platys*, *A. phagocytophilum, Ehrlichia canis*, *Babesia vogeli, Hepatozoon canis, Rickettsia conorii;* most of them not yet reported in Hungary as autochthonous infections.

Objectives: The study aims at (i) the genetic identification of *R. sanguineus* ticks collected from domestic dogs in central Hungary using nuclear and mitochondrial genetic markers, and (ii) pathogens potentially transmitted by *R. sanguineus* in this recently established focus.

Material and methods: In 2020–2021, a total of 1839 ticks collected by veterinarians from dogs and cats were received from Hungary. All the ticks were morphologically identified using standard morphological keys; 169 individuals were identified as *R. sanguineus*. A subset of 15 *R. sanguineus* was chosen for photographing and molecular analysis of three markers (16S rDNA, 12S rDNA, Cox1). All 169 ticks identified as *Rhipicephalus* were tested for the presence of *A. platys* and *E. canis* by nested PCR, targeting a 351-bp portion of the 16S rRNA gene and 389-bp of the 16S rRNA gene, respectively.

Results: In the selected 15 *R. sanguineus* specimens, obtained sequences of 16S rDNA, 12S rDNA, and Cox1 invariably clustered into a single haplotype in phylogenetic analyses (maximum likelihood), all belonging to *R. sanguineus* s.str. clade. This clade was well separated from *R. linnaei* (formerly the tropical lineage) and from other species within the complex, always supported by the high bootstrap value. Of 169 *R. sanguineus* s.str. ticks none were positive for *A. platys* and *E. canis*. However, sequencing of the amplicon for *A. platys* of the expected size from one dog revealed the presence of *A. phagocytophilum* (the agent of human granulocytic anaplasmosis).

Conclusion: *R. sanguineus* is not an indigenous tick in Hungary, its autochthonous presence was first observed in 2005, and it has been rarely found since. In our study, we confirmed a persistence of *R. sanguineus* population previously reported in 2016 and identified found ticks as *R. sanguineus* s.str. However, we did not prove the occurrence of tested pathogens associated with this tick species. Further work is needed to investigate also other pathogens transmitted by the brown dog tick, including *B. vogeli*, *H. canis,* and *R. conorii* in known Hungarian populations of this tick.

Keywords (7)
Rhipicephalus sanguineus species complex, tick-borne pathogens, *Anaplasma platys*, *Ehrlichia canis*, *Babesia vogeli*, *Hepatozoon canis*, *Rickettsia conorii*

P 097

High diversity of tick borne agents in ticks collected from farm animals in Vrbovce (Western Slovakia)

V. Rusňáková Tarageľová[1], *B. Mangová*[1], *Y. M. Didyk*[1], *D. Selyemová*[1], *M. Chvostáč*[1], *M. Derdáková*[1]
[1]Slovak Academy of Sciences, Zoology, Bratislava, Slovakia

Ticks transmit a wide range of pathogenic microorganisms. Many species of vertebrates were found to be infested by ticks. Animals provide the inevitable blood meal for ticks, but some species are also involved in the maintenance of tick-borne pathogen cycles as reservoir or amplifying hosts. Moreover, vertebrates can serve as sentinels for monitoring the occurrence of ticks and the risk of TBD in the nature. Ticks were collected at farm near Vrbovce, Myjava, Slovakia. DNA was isolated using alkaline hydrolysis and/or by using commercially available kits. To study the genetic variability of tick-borne microorganisms, PCRs methods and further DNA sequencing were used. Ticks (n = 145) from various hosts (cats, hedgehog, goats, dogs, horses, sheep, rabbit) were analyzed. Feeding *Ixodes ricinus* (n = 139) and *Dermacentor reticulatus* (n = 15) were collected. In *I. ricinus* ticks *Borrelia burgdorferi* sensu lato (9.0%) with the dominance of *B. afzelii*, *Borrelia miyamotoi* (0,7%), *Rickettsia* spp. (9.0%) with the species *Rickettsia helvetica* (53.8%) and *Rickettsia monacensis* (23.1%), *Anaplasma phagocytophilum* (40.4%), *Babesia* spp. (4.1%) with *Babesia microti* (33.3%), *Babesia venatorum* (33.3%) and *Babesia capreoli* (16.7%) were detected. In *D. reticulatus*, *Rickettsia raoulti* was confirmed (2.1%). In *I. ricinus* ticks collected from vegetation (n = 165) *B. burgdorferi* s.l. (24.8%), *B. miyamotoi* (3.0%) and *Rickettsia helvetica* (3.6%) were detected. Due to the high number as well as diversity of detected zoonotic pathogens transmitted by ticks, it is necessary to pernamently monitor and survey areas where domestic animals are kept in close proximity to wildlife.

Acknowledgement: This study was financially supported by the Scientific Grant Agency of Ministry of Education and Slovak Academy of Sciences, by the project VEGA no. 2/0137/21 and 2/0004/22.

P 098

Antibody-mediated blunting of tick salivary enzymatic “cutlery”

D. Foitlova[1,2]*, T. Hatalova*[1,2]*, Z. Berankova*[2]*, J. Perner*[1]
[1]Biology Centre, Czech Academy of Sciences, Institute of Parasitology, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic

Introduction: Ticks salivate while feeding on their vertebrate hosts. Saliva helps tick blood-feeding through interaction and modulation of host anti-hemostatic and immunomodulatory components. Although most of the proteinaceous fraction of tick saliva is of little immunogenicity, repeated feeding of ticks on mammalian hosts leads to the formation of a few types of anti-tick antibodies and leads to a decrease of the tick blood-feeding performance.

Objectives: We sought to identify immunogenic proteins of *Ixodes ricinus* saliva that are exposed to the rabbit immune system by repetitive full feeding of adult *I. ricinus* females. The function of the raised antibodies was then evaluated.

Materials & methods: To identify tick salivary antigens, we isolated immunoglobulins from rabbits that had been previously exposed to tick feeding and used the immunoglobulins for a pull-down of antigens from tick saliva.

Results: The most dominant salivary immunogens of ticks (*I. ricinus*), identified in our study, were zinc-dependent metalloproteases of three different families. To corroborate the role of metalloproteases at the tick/host interface, we fed ticks micro-injected with a zinc metalloprotease inhibitor, phosphoramidon, on a rabbit. These ticks clearly failed to initiate their blood-feeding. However, when ticks were fed *ex vivo* on the membrane feeding system, neither feeding the “immune blood” of repeatedly infested rabbits, nor phosphoramidon injection into ticks, prevented their engorgement, clearly indicating that Zn metalloproteases play a decisive role in the success of tick *in vivo* feeding (PMID: 33312963).

Conclusion: We argue that long (days) association of ticks with their host enables immune detection of their salivary components and that neutralization of the identified salivary metalloproteases may facilitate an “antibody-mediated blunting of tick salivary cutlery”, preventing tick infestations and possibly also the transmission of tick-borne pathogens.

O 058

Dynamics of ticks and tick-borne diseases in different ecosystems in an endemic area of France

N. Boulanger[1], *M. L. Poulle*[2], *C. Barthel*[1], *R. Rivera*[1], *A. Haidar-Ahmad*[1]
[1]University of Strasbourg, Bacteriology, Strasbourg, France
[2]CERFE, Ecology, Reims, France

Introduction: Tick population dynamics and the circulation of tick-associated pathogens are strongly impacted by the environment and human activities. The French National Centre for Scientific Research (CNRS) has created 14 geographical regions in France to facilitate the development of collaborative interdisciplinary research projects with local stakeholders like forest workers, hunters, naturalists and others. **Objectives**: We have selected the Argonne region (East of France) to develop a research project where we study the impact of human practices and socio-ecosystems on tick-borne diseases. **Materials & methods**: Three well-defined ecosystems were selected in the Argonne region: a fenced wildlife park, two wetland sites and three forested areas. *Ixodes ricinus* and *Dermacentor* spp. were collected by flagging on vegetation during their activity peak between March and June 2021 and 2022. Tick-borne pathogens were detected in ticks by real-time PCR. **Results**: The PCR revealed, at various densities between the sites, the presence of *Borrelia burgdorferi* sensu lato complex, *Anaplasma phagocytophilum*, *Borrelia miyamotoi* and *Neoehrlichia mikurensis* in *Ixodes* ticks. One site (the “deer park”), a fenced site of regenerating forest, showed a very high density of *I. ricinus* and particularly of infected nymphs*. Borrelia burgdorferi* s.l. (*B. afzelii* > B. *garinii* > *B. valaisiana* > *B. burgdorferi* s.s.) and *N. mikurensis* were the two most prevalent pathogens in this site compared to the other sites (forest and wetlands). **Conclusion**: The “deer park” highlights the importance of the wildlife in the circulation of tick-associated pathogens and its essential role in maintaining tick populations. This ecosystem is an outstanding example of an “ideal” site for ticks and tick-borne diseases. Interestingly, *Dermacentor* was not collected in this site; whereas it was found in the other ecosystems.

O 059

Vector-borne disease risk – a comprehensive analysis at the human-wildlife interface

K. Hollett[1], *V. Millien*[1]
[1]McGill University, Biology, Montreal, Canada

Tick-borne diseases are of increasing concern to recreationalists spending time in nature parks. Human presence may indirectly impact tick populations via disturbances of wildlife host species. In Eastern North America white-tailed deer (*Odocoileus virginianus*) are an overabundant host species, essential for the establishment and maintenance of blacklegged tick (*Ixodes scapularis*) populations. Deer behavior such as vegetation browsing can have significant environmental impacts. As black-legged ticks carry the causal agent of Lyme disease (*Borrelia burgdorferi s.l.*), understanding the dynamics between habitat types can improve predictions on the distribution of both disease and vector. Field

surveys of a nature reserve that has distinct areas of high and low human usage were conducted to determine if recreation is indirectly affecting the distribution of ticks by altering the distribution of deer, therefore influencing habitat quality. Data on human presence, deer distribution, vegetation, and tick density were collected across public open access and private conservation sectors of the reserve. It was expected that the magnitude of human presence in a nature reserve would affect deer distribution, leaving areas less frequented by deer with a preferential habitat for ticks. Preliminary results suggest there is a considerable decrease in vegetation due to deer browsing, a temporal avoidance behavior of deer to human recreationalists, and a substantial difference in tick abundance across areas of high and low human presence. Understanding the complex interactions of ticks, tick hosts, and humans are essential for predicting variation in Lyme disease risk on a local scale and refining mitigation strategies.

O 060

Potassium ion channels represent a novel target to inhibit tick blood feeding and pathogen transmission

D. Swale[1], *A. Mulenga*[2], *K. Macaluso*[3]
[1]University of Florida, Emerging Pathogens Institute, Dept of Entomology, Gainesville, FL, United States
[2]Texas A&M University, Veterinary Pathobiology, College Station, United States
[3]University of South Alabama College of Medicine, Microbiology and Immunology, Mobile, AL, United States

Background: Salivary gland secretions are essential for tick feeding and thus, reducing or preventing saliva secretions into the vertebrate host is likely to reduce feeding and hinder pathogen life cycles. Unfortunately, the membrane physiology of tick salivary glands is underexplored and this gap in knowledge limits the development of novel therapeutics for inducing cessation of tick feeding. We aimed to test the influence of inward rectifier potassium (Kir) channel subtypes to the functional capacity of the isolated tick salivary gland and as a target to inhibit tick blood feeding.
Methods: We studied the influence of Kir channels to the functional capacity of the tick salivary gland using a modified Ramsay assay that allowed for pharmacological activation or inhibition of Kir channels. We quantified changes to the secreted proteome though PEAKS LFQ to examine differences in protein abundance, LC-MS/MS, and standard Western blotting against salivary proteins known to be relevant to feeding or pathogen transmission.
Results: Pharmacological activation of Kir channels led to near inhibition of fluid secretion from isolated salivary glands that was directly correlated with a 99% reduction of blood ingestion during feeding. Further, significant changes to secreted proteins were documented after pharmacological modulation of Kir channels with near elimination of secretion of AV422, AAS27, and AAS41. These three proteins have key roles in blood feeding and/or pathogen transmission. Importantly, ingestion of Kir modulators during blood feeding induced mortality in less than 12 hours that is likely resultant from altered osmoregulatory capacity.
Conclusion: These data indicate modulation of salivary gland specific ion channels with small-molecule chemistry can inhibit fluid- and protein-secretion in saliva to inhibit blood feeding and pathogen transmission, which provides an alternative to development of anti-tick or anti-pathogen vaccines.

O 061

Targeting canonical metabolic pathways – a novel strategy for tick control

P. Kopáček[1], D. Sojka[1], R. Šíma[1], M. Kučera[1,2], T. Kozelková[1,2], S. Kropáčková[1,2], L. Robbertse[1]
L. Grunclová[1], J. Perner[1]
[1]Biology Centre of the Czech Academy of Sciences, Parasitology, České Budějovice, Czech Republic
[2]University of South Bohemia, Faculty of Sciences, České Budějovice, Czech Republic

The rational approach to finding effective strategies to control ticks and the diseases they transmit has traditionally focused on tick-specific adaptations to their parasitic lifestyle and major physiological differences from their hosts. However, several recent examples from other parasites show that targeting near-canonical processes between parasites and their hosts could surprisingly lead to the discovery of effective antiparasitic agents. This requires a sufficient breadth of the therapeutic window between toxicity to the parasite and host.

Inspired by these results, we used RNAi-mediated silencing or chemical inhibition of selected molecules by artificial feeding to investigate the potential of targeting relatively conserved physiological processes and metabolic pathways in our model tick, *Ixodes ricinus*.

We confirmed that RNAi-based interventions in tyrosine degradation, elimination of selected amino acid-tRNA synthetases, insulin signaling pathway components, cholesterol, lipid uptake, chemical inhibition of the tick proteasome, or the use of antibody-based acaricides exert quite striking phenotypes on tick development or mortality.

These promising results stimulate and shape our future research efforts to find new potential targets for efficient anti-tick preparations or vaccines.

Acknowledgment: *Supported by GACR* 21-08826S

O 062

Proteasome inhibitors effectively combat ticks as well as transmitted pathogens

L. Robbertse[1], P. Fajtová[2], P. Šnebergerová[1,3], A. J. O'Donoghue[2,4], D. Sojka[1]
[1]Biology Centre of the Czech Academy of Sciences, Parasitology, Ceske Budejovice, Czech Republic
[2]University of California, San Diego, Skaggs School of Pharmacy and Pharmaceutical Sciences, La Jolla, CA, United States
[3]University of South Bohemia, Faculty of Sciences, České Budějovice, Czech Republic
[4]University of California, San Diego, Medicine, La Jolla, CA, United States

Introduction: Proteasomes are large protein complexes serving as the degrading arm of the proteasome-ubiquitin system in eukaryotic cells. Selective inhibitors of the 20S catalytic core proteases of parasites over host proteasomes has been validated as one of the most relevant novel therapeutic strategies for infectious diseases including malaria, leishmaniasis, schistosomiasis and Chagas disease. Our mission is the validation of selective proteasome inhibition as a novel intervention strategy to reduce the burden of tick-borne diseases.

Objectives: Herein we test the hypothesis that both the tick and the tick-borne Babesia proteasomes can be selectively targeted.

Materials & Methods: We demonstrate the massive effect of FDA-approved proteasome inhibiting drugs and novel carmaphycin B-based proteasome inhibitors on the propagation of two tick-borne *Babesia* species, *B. divergens* (ex vivo) and *B. microti* (in vivo) and also on the survival and fitness *Ixodes ricinus* ticks via membrane feeding on inhibitor containing blood. This includes the demonstration of the on-target effect using Western blot of polyubiquitinylated proteins and fluorescent peptidyl substrate enzymatic assays.

Results: We validate *Babesia* proteasome as a suitable target for the yet missing specific therapy using commercially available inhibitors, and we further identified compounds with increased selectivity index for *Babesia* over host proteasome by screenings of compounds primarily developed to target the proteasome of the *Babesia*-related *P. falciparum*, mainly based on the carmaphycin B scaffold. Additionally, some of the commercial proteasome inhibitors have been also found effective against Lyme borreliosis and surprisingly, even against the tick vectors themselves.

Conclusions: Herein we demonstrate the universally applicable potential of selective proteasome inhibition that can be applied to combat arthropod ticks as well as tick-transmitted pathogens.

BAVARIAN NORDIC.
SPREADING HOPE.
PIONEERING VACCINES.

Date of preparation February 2023 | Job code GL-GEN-2300005
Bavarian Nordic A/S, Philip Heymans, Alle 3 2900 Hellerup, Denmark.

www.conventus.de

© Nejron Photo / Fotolia

Success through
versatile
solutions

As a full-service PCO, we provide you with intelligent and innovative solutions in an advisory and implementing manner.