13TH INTERNATIONAL SYMPOSIUM ON TICKS AND TICK-BORNE DISEASES

28–30 March 2019 ::: Leonardo Hotel Weimar, Germany

www.ittbd-symposium.com

DGP 2020 BONN

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Prof. Dr. Achim Hörauf & Team

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Dear Colleagues,

It is a great pleasure to welcome you to the 13th International Symposium on Ticks and Tick-borne Diseases (ISTTBD-XIII) in Weimar. This symposium series was organized for the first time in 1991 after the fall of the Iron Curtain as the "Potsdam Symposium on Tick-borne Diseases" and has since been a meeting ground for scientists studying ticks and tick-borne diseases from the East and West in an informal setting. The symposium took place biannually until 2013 and we have been very happy to be able to organize it again this year under the auspices of the German Society for Parasitology (DGP) in the beautiful city of Weimar.

The city of Weimar has as many as three entries in the UNESCO's World Heritage List: Classical Weimar fronting 16 buildings and architectural ensembles, most of which are within walking distance of the Leonardo Hotel, three Bauhaus buildings and the Memory of the World, which contains handwritten manuscripts of Johann Wolfgang von Goethe. We do hope that you find time to visit some of these sites.

We are pleased with the large number of delegates from over 30 countries that will present their research at ISTTBD-XIII and would also like to thank the sponsors for their generous support. We are eagerly looking forward to three days of exciting presentations and discussions and hope that all of you will have a successful and pleasant meeting.

Dr. Olaf Kahl

Prof. Dr. Ard Nijhof

Prof. (a. D.) Dr. Jochen Süss

Thursday, 28 March

Goethesaal

10:00–13:00
Registration & poster mounting
Belvedere I-IV and Foyer
p. 8

13:00–13:20
Conference opening p.8

13:20–13:50
Keynote 1

13:50–15:20
Session 1 I TBE 1
p. 8

15:50–17:20
Session 2 I Sinnecker Kunz Award
p. 9

17:20–18:45
Session 3 I Ticks and others I
p. 10

19:30–21:30
Welcome reception
Belvedere I-IV and Foyer
p. 28

Friday, 29 March

Goethesaal

09:00–09:30
Keynote 2 p. 11

09:30–10:25
Session 4 I Lyme Borreliosis I Klaus Kurtenbach Session
p. 11

12:20–13:10
Session 5 I Lyme Borreliosis II
p. 11

14:10–14:40
Keynote 3 p. 12

14:40–15:45
Session 6 I Tick-borne pathogens I
p. 12

16:15–17:05
Session 7 I Tick-borne Encephalitis II p. 13

17:05–17:45
Session 7.1 I Lyme Borreliosis III p. 13

Belvedere III-IV

16:15–17:30
Session 7.2 I Ticks and others II
p. 14

Belvedere I-IV and Foyer

10:50–12:20
P1
Poster session 1
p. 17

19:00–00:00
Gala dinner
p. 28

Saturday, 30 March	
Goethesaal	Belvedere I-IV and Foyer
09:00–09:30	
Keynote 4 p. 15	
09:30–11:00	
Session 8 I Ticks and others III p. 15	
	11:30–13:00
	P2 **Poster session 2** p. 22
13:00–13:30	
Keynote 5 p. 16	
13:30–14:10	
Session 9 I Tick-borne pathogens II p. 16	
14:10–15:00	
Closing address and awards p. 16	

Legend

- Registration, opening of conference, closing ceremony
- Keynote session
- Short lectures
- Social programme
- Poster session

Notes

10:00–13:00 Registration & Poster mounting (Belvedere I–IV & Foyer)

12:00–13:00 Lunch snack (Belvedere I–IV & Foyer)

13:00–13:20 Address conference chairs (Goethesaal)

Goethesaal

Welcome note by conference chairs
Ard Nijhof (Berlin/DE), Jochen Süss (Renthendorf/DE), Olaf Kahl (Berlin/DE)

13:20–13:50 Keynote 1

Goethesaal
Chairs: Ard Nijhof (Berlin/DE), Jochen Süss (Renthendorf/DE)

13:20 O1 Changing paradigms for tick-borne rickettsioses in North America
Christopher D. Paddock (Atlanta, GA/US)

13:50–15:20 Session 1 – TBE 1

Goethesaal
Chairs: Oxana Belova (Moscow/RU), Ute Mackenstedt (Stuttgart/DE)

13:50 O2 Tick-borne encephalitis virus of Western and Eastern Siberia: genetic diversity, geographical distribution and evolution
Sergey Tkachev (Novosibirsk/RU)

14:05 O3 Properties of the tick-borne encephalitis virus population during acute and persistent infection of ixodid ticks
Oxana Belova (Moscow/RU)

14:15 O5 Temporal and spatial epidemiology of human tick-borne encephalitis in Germany from 2016 to 2018
Gerhard Dobler (Munich, Stuttgart/DE)

14:30 Discussion

14:40 O6 Phylogeny of TBE virus in Austria and Central Europe
Malena Bestehorn (Stuttgart/DE)

14:50 O7 Detection of tick-borne encephalitis virus antibodies in sera of sheep and goats in Mecklenburg-Western Pomerania (north-eastern Germany)
Silvius Frimmel (Rostock/DE)

15:00 O8	Ixodid ticks and tick-borne encephalitis virus in the Republic of Tuva Ivan Kholodilov (Moscow/RU)
15:10	Discussion
15:20–15:50	Coffee break (Belvedere I–IV & Foyer)
15:50–17:20 Goethesaal	Session 2 – Sinnecker Kunz Award session
Chairs	Christopher D. Paddock (Atlanta, GA/US), Daniel Ruzek (Brno, České Budějovice/CZ)
15:50	Introduction Sinnecker Kunz Award
16:00 O9	Canine anti-α-Gal antibodies: Potential role in red meat allergy and protection against tick-borne pathogens Adnan Hodzic (Vienna/AT)
16:10 O10	Feeding-induced salivary gland genes of *Ixodes ricinus* ticks as targets for anti-tick vaccines Abhijeet Nayak (Amsterdam/NL)
16:20 O11	Interpopulation differences in behavior of ixodid ticks Alexandra Evgenievna Polienko (Moscow/RU)
16:30 O12	Dynamics of the tick pathobiome Emilie Lejal (Maisons-Alfort/FR)
16:40 O13	The *Ixodes ricinus* salivary gland proteome during feeding and *Borrelia* infection Michelle Klouwens (Amsterdam/NL)
16:50 O14	The factors that shape vector aggregation on small mammal hosts Alexandra Lawrence (Bayreuth/DE)
17:00	Discussion

17:20–18:45 Session 3 – Ticks and others I

Goethesaal

Chairs Cornelia Silaghi (Greifswald – Insel Riems/DE), Thomas Jaenson (Uppsala/SE)

17:20 O15 Does tick immune system fight with tick-transmitted pathogens?
Ondrej Hajdusek (České Budějovice/CZ)

17:30 O16 *Rhipicephalus rossicus*: overview of a neglected tick knocking at the door of Europe
Gianluca D'Amico (Cluj-Napoca/RO)

17:40 O17 Identification and characterization of catalase from the hard tick *Haemaphysalis longicornis* and evaluation of its *in vitro* antioxidant activity
Kodai Kusakisako (Sapporo/JP)

17:50 Discussion

18:05 O18 Comparison of tick diversity between adult and juvenile red foxes
Dorota Dwużnik (Warsaw/PL)

18:15 O19 The distribution limit of *Ixodes ricinus* in north-western Europe
Dag Hvidsten (Bodø/NO)

18:25 O20 Isolation and propagation in tick cell lines of *Spiroplasma* spp. from European *Ixodes* and *Dermacentor* ticks
Lesley Bell-Sakyi (Liverpool/GB)

18:35 Discussion

19:30–21:30 Welcome reception (Belvedere I–IV & Foyer)
(see page 28)

09:00–09:30 **Keynote 2**

Goethesaal

Chairs — Gabriele Margos (Oberschleißheim/DE), Olaf Kahl (Berlin/DE)

09:00 O21 — *Rhipicephalus sanguineus* group: an update of the taxonomic status and its epidemiological implication
Santiago Nava (Santa Fe/AR)

09:30–10:25 **Session 4 – Lyme Borreliosis I – Klaus Kurtenbach session**

Goethesaal

Chairs — Gabriele Margos (Oberschleißheim/DE), Olaf Kahl (Berlin/DE)

09:30 — Introduction: Lyme Borreliosis I – Klaus Kurtenbach session

09:35 O22 — Termination of alternative pathway activation by binding of Factor H and FHL-1 facilitates complement resistance of *Borrelia mayonii*
Peter Kraiczy (Frankfurt a. M.)

09:50 O23 — Core genome phylogenetic analysis of the avian-associated *Borrelia turdi* indicates a close relationship to *Borrelia garinii*
Gabriele Margos (Oberschleißheim/DE)

10:00 O24 — Importance of birds in the circulation of neuroinvasive strains of *B. garinii*
Marketa Derdakova (Bratislava/SK)

10:15 — Discussion

10:25–10:50 — Coffee break (Belvedere I–IV & Foyer)

10:50–12:20 **Poster session 1**
(for more details see page 17)

12:20–13:10 **Session 5 – Lyme Borreliosis II**

Goethesaal

Chair — Marketa Derdakova (Bratislava/SK), Peter Kraiczy (Frankfurt a. M./DE)

12:20 O25 — Host-parasite interactions between *Borrelia burgdorferis* .l. and its avian reservoir hosts
Ana Claudia Norte (Coimbra, Lisboa/PT)

12:35 O26	The genus *Borrelia* reloaded Gabriele Margos (Oberschleißheim/DE)
12:50 O27	Phyloproteomic and functional analyses do not support a split in the genus *Borrelia* (phylum Spirochaetes) Agustín Estrada-Peña (Zaragoza/ES)
13:00	Discussion
13:10–14:10	Lunch snack (Belvedere I–IV & Foyer)
14:10–14:40 Goethesaal	Keynote 3
Chairs	Michael Levin (Atlanta, GA/US), Snorre Stuen (Sandnes/NO)
14:10 O28	Molecular mechanisms of inhibition of tick-borne encephalitis virus by nucleoside analogues and neutralization by a monoclonal antibody Daniel Ruzek (Brno, České Budějovice/CZ)
14:40–15:45 Goethesaal	Session 6 – Tick-borne pathogens I
Chairs	Michael Levin (Atlanta, GA/US), Snorre Stuen (Sandnes/NO)
14:40 O29	Pathogenic potential of tick-borne symbiotic *Rickettsia* species Michael Levin (Atlanta, GA/US)
14:50 O30	*Phlebovirus*-like sequences detected in ticks and mosquitoes collected in the Russian Federation Alexander S. Klimentov (Moscow/RU)
15:00 O31	Zoonotic potential of species in the genera *Anaplasma* and *Ehrlichia* Snorre Stuen (Sandnes/NO)
15:10	Discussion
15:20 O32	Investigating small and medium-sized mammals to identify potential reservoirs of *Borrelia miyamotoi* in the North Central U.S.A. Seungeun Han (East Lansing, MI/US)
15:30 O33	The Tick-Borne-Diseases STING-study – Clinical outcome, epidemiology and prevalence of tick-borne pathogens in Scandinavia Per-Eric Lindgren (Linköping/SE)

15:40	Discussion
15:45–16:15	Break (Belvedere I–IV & Foyer)
16:15–17:05	Session 7 – Tick-borne Encephalitis II
Goethesaal	
Chairs	Sergey Tkachev (Novosibirsk/RU), Gerhard Dobler (Munich, Stuttgart/DE)
16:15	Can TBE lead to neurodegeneration – a résumé
O34	Anna Moniuszko-Malinowska (Białystok/PL)
16:25	Risk factors and tick-borne encephalitis spatio-temporal variation. A case study in
O35	northern Italy Valentina Tagliapietra (San Michele all'Adige/IT)
16:35	Discussion
16:40	Tick-borne encephalitis in Siberia: statistics, incidence, association of the disease
O36	severity and virus genotype Nina Tikunova (Novosibirsk/RU)
16:50	Host genetic control of tick-borne encephalitis virus infection: current progress and
O37	future prospects Andrey Barkhash (Novosibirsk/RU)
17:00	Discussion
17:05–17:45	Session 7.1 – Lyme Borreliosis III
Goethesaal	
Chairs	Joppe Hovius (Amsterdam/NL), Andrei Daniel Mihalca (Cluj-Napoca/RO)
17:05	Tracking the route of *Borrelia afzelii* transmission from infected *Ixodes ricinus* nymphs
O38	Radek Sima (České Budějovice/CZ)
17:15	Phenology of *Ixodes ricinus* and its infection rates with *Borrelia burgdorferi* in the
O39	Netherlands; results of ten years of monthly collections Nienke Hartemink (Wageningen/NL)
17:25	A pan-European overview on the surveillance, reporting, data availability and
O41	laboratory capacity for Lyme borreliosis Andrei Daniel Mihalca (Cluj-Napoca/RO)
17:35	Discussion

16:15–17:30	Session 7.2 – Ticks and others II
Belvedere III–IV	
Chairs	Santiago Nava (Santa Fe/AR), Annetta Zintl (Dublin/IE)
16:15	Genetic variability of *Dermacentor reticulatus* in Europe
042	Algimantas Paulauskas (Kaunas/LT)
16:25	Population genetics of *Dermacentor variabilis* in the United States
043	Paula Lado (Columbus, OH/US)
16:35	Discussion
16:40	Identification of closely related *Ixodes* species by protein profiling with MALDI-TOF
044	Mass Spectrometry. Pierre Boyer (Strasbourg/FR)
16:50	Development of a biological tick trap based on an attract-and-kill strategy:
046	efficacy to attract and catch ticks Kerstin Büchel (Berlin/DE)
17:00	Discussion
17:05	Comparative efficacy of natural and synthetic acaricides against larvae of *Rhipi-*
047	*cephalus sanguineus* Canestrini (Acari: Ixodidae) Gervasio Bechara (Curitiba/BR)
17:15	*Hemolivia mauritanica* infection in *Hyalomma aegyptium* from Corum Province, Turkey
048	Gönül Arslan Akveran (Çorum/TR)
17:25	Discussion
19:00–00:00	Gala Dinner (Villa Haar)
	(see page 28)

09:00–09:30 Keynote 4

Goethesaal

Chairs: Lidia Chitimia-Dobler (Munich, Stuttgart/DE); Alejandro Cabezas-Cruz (Maisons-Alfort/FR)

09:00 O50 Eco-Epidemiology of Crimean-Congo Hemorrhagic Fever (CCHF)
Zati Vatansever (Kars/TR)

09:30–11:00 Session 8 – Ticks and others III

Goethesaal

Chairs: Lidia Chitimia-Dobler (Munich, Stuttgart/DE); Alejandro Cabezas-Cruz (Maisons-Alfort/FR)

09:30 O51 Ticks and tick-borne pathogens from migratory birds in southern and central Europe
Georg Duscher (Vienna/AT)

09:40 O52 *Hyalomma marginatum* and *Hyalomma rufipes*: What do we know about the biology and ecology of these regular visitors in central Europe?
Olaf Kahl (Berlin/DE)

09:50 O53 Molecular species discrimination of *Hyalomma* and other ticks potentially transmitting Crimean-Congo hemorrhagic fever virus in sub-Saharan Africa
Ansgar Schulz (Greifswald – Insel Riems/DE)

10:00 Discussion

10:10 O54 TBE in Germany: Design and first results of the project OSWALD
Franz Rubel (Vienna/AT)

10:20 O55 Predicting the TBE and Lyme borreliosis vector *Ixodes ricinus* in space and time
Katharina Brugger (Vienna/AT)

10:30 Discussion

10:35 O56 Tick-bite associated dermatitis in an Afro-descendant population living in a tropical region in South West Colombia
Niklas Weber (Berlin/DE)

10:45 O57 Why ticks and tick-borne pathogens produce their own α-Gal?
Alejandro Cabezas-Cruz (Maisons-Alfort/FR)

10:55 Discussion

11:00–11:30	Coffee break (Belvedere I–IV & Foyer)
11:30–13:00	Poster session 2 (for more information see page 22)
13:00–13:30	Keynote 5
Goethesaal Chairs	Georg Duscher (Vienna/AT), Hein Sprong (Utrecht/NL)
13:00 O58	Unlocking the mechanisms of tick salivary gland control, promoting the development of tick and tick-borne disease control measures Ladislav Simo (Maisons-Alfort/FR)
13:30–14:10	Session 9 – Tick-borne pathogens II
Goethesaal Chairs	Georg Duscher (Vienna/AT), Hein Sprong (Utrecht/NL)
13:30 O59	Tick mischief: What can be detected in juvenile ticks *Dermacentor reticulatus* collected from rodents? Anna Bajer (Warsaw/PL)
13:40 O60	Babesiosis in a Northern German cattle herd – epidemiological investigations Andrea Springer (Hanover/DE)
13:50 O62	Typing of *Anaplasma phagocytophilum* strains by multilocus sequences typing (MLST), *ankA* gene sequencing and presence or absence of the *drhm* gene Friederike von Loewenich (Mainz/DE)
14:00	Discussion
14:10–15:00	Closing address & awards
Goethesaal	
	Closing address conference chairs Ard Nijhof (Berlin/DE), Jochen Süss (Renthendorf/DE), Olaf Kahl (Berlin/DE)

Please see page 31 for general poster session information.

10:50–12:20 Poster session 1 | Belvedere I–IV & Foyer

Lyme Borreliosis | LB-1 – LB-17

LB-1 Crystal structure of BB0365 from Lyme disease agent *Borrelia burgdorferi*
Kalvis Brangulis (Riga/LV)

LB-3 NMR structural analysis of decorin-binding protein A from *Borrelia afzelii*, the main factor of virulence
Libor Hejduk (České Budějovice/CZ)

LB-5 Population structure of *Borrelia turcica* from Greece and Turkey
Gabriele Margos (Oberschleißheim/DE)

LB-7 *Borrelia maritima* sp. nov., a novel genospecies of the *B. burgdorferi* sensu lato complex, occupies the most basal position in the North American clade
Gabriele Margos (Oberschleißheim/DE)

LB-11 Cervical myelitis as uncommon manifestation of neuroborreliosis – a case report
Barbara Oczko-Grzesik (Bytom/PL)

LB-13 Longitudinal study of infection with *Borrelia* spp. in questing *Ixodes ricinus* from northwestern Spain.
Susana Remesar (Lugo/ES)

LB-15 Prevalence and genetic classification of *Borrelia burgdorferi* sensu lato in different study sites in Slovakia
Diana Selyemová (Bratislava/SK)

LB-17 Detection of *Borrelia burgdorferi* sensu lato in a recently established population of the taiga tick, *Ixodes persulcatus* in Sweden
Peter Wilhelmsson (Linköping/SE)

LB-19 Altered gene expression upon infection with *Borrelia afzelii* in nymphal *Ixodes ricinus* salivary glands during feeding.
Jos Trentelman (Amsterdam/NL)

Tick-borne pathogens | TBP-1 – TBP-37

TBP-1 Molecular identification and characterization of *Theileria* spp. responsible for ovine theileriosis in Egypt
Amira Abdel Aleem AL-Hosary (Assiut/EG)

TBP-2 Genome sequencing of a British ovine isolate of *Anaplasma phagocytophilum* and proteomic analysis of p44 expression in tick cells in vitro
Alaa M. Al-Khafaji (Liverpool/GB)

TBP-3 The assessment of the risk of *Coxiella burnetii* and *Rickettsia* spp. infections in north-eastern Poland
Anna Moniuszko-Malinowska (Białystok/PL)

TBP-5 The role of different ungulate species in the ecology of tick-borne diseases
Nannet Fabri (Umea/SE; Utrecht/NL)

TBP-7 Implementation of the DAMA protocol: mitigation of infection risk with emerging tick-borne zoonotic bacteria in cities
Gabor Foldvari (Tihany, Budapest/HU)

TBP-9 Case of *Dermacentor*-borne necrosis erythema lymphadenopathy (DEBONEL) imported from Spain
Peter Hagedorn (Berlin/DE)

TBP-11 Epidemiological investigation of Crimean-Congo hemorrhagic fever virus foci among livestock in the endemic region of Pakistan
Khushal Khan Kasi (Greifswald – Insel Riems/DE)

TBP-13 Abundance of ticks and tick-borne bacterial pathogens in northwestern Germany
Steffen Knoll (Hanover/DE)

TBP-15 Trends of tick-borne pathogens in small mammal and ticks from Saxony, Germany
Nina Krol (Leipzig/DE)

TBP-17 First report of *Babesia microti* and *B. canis* in hedgehog blood
Justyna Liberska (Poznań/PL)

TBP-19 Occurrence of tick-borne pathogens in Lithuanian rodent communities
Dalyte Mardosaite-Busaitiene (Kaunas/LT)

TBP-21 Turkey tick news: preliminary results from a molecular investigation into the presence of tick-borne pathogens in host-seeking ticks in Anatolia
Ömer Orkun (Ankara/TR)

TBP-23 Prevalence and zoonotic potential of *Anaplasma phagocytophilum* in roe deer from Spain
Susana Remesar (Lugo/ES)

TBP-25 First record of *Rickettsia vini* in *Ixodes lividus* ticks from sand martin (*Riparia riparia*) nests in Lithuania
Jana Radzijevskaja (Kaunas/LT)

TBP-27 Combination of microbiome analysis and serodiagnostics to assess the risk of pathogen transmission by ticks to humans and animals in central Germany
Volkhard Kempf (Frankfurt a. M./DE)

TBP-31 Molecular detection of tick-borne bacteria in questing *Ixodes ricinus* ticks in Northern Italy
Fausta Rosso, Valentina Tagliapietra (San Michele all'Adige/IT)

TBP-33 Vector-borne parasites in Namibian cheetahs (*Acinonyx jubatus*) and leopards (*Panthera pardus*)
Maria Serocki (Berlin/DE)

TBP-37 Alkhurma hemorrhagic fever virus RNA in *Hyalomma* ticks infesting migratory birds
Tove Hoffmann (Uppsala/SE)

Ticks & Others | T&O-1 – T&O-35

T&O-1 Expansion and control of the tick *Dermacentor reticulatus* in Poland
Anna Bajer (Warsaw/PL)

T&O-3 New records of *Compluriscutula vetulum* larvae in Burmese amber, with notes on morphology
Lidia Chitimia-Dobler (Munich, Stuttgart/DE)

T&O-5 *Ixodes ricinus* salivary serpin IRS-1 as a modulator of the host immune response
Adéla Chlastáková (České Budějovice/CZ)

T&O-7 The Ixogon® Zeckenrollen – a field trial to test the efficacy of a tick control system
Marco Drehmann (Stuttgart/DE)

T&O-9 Study of the life cycle of *Hyalomma excavatum* and *Hyalomma scupense* under laboratory conditions
Khawla Elati (Ariana/TN)

T&O-13 Occurrence of *Ixodes inopinatus* in Northern Germany and prevalence of tick-borne pathogens
Christina Strube (Hanover/DE)

T&O-15 Detection of questing *Ixodes frontalis* larvae in a forest close to Berlin (Germany) in November 2018
Olaf Kahl (Berlin/DE)

T&O-19 Structural and functional characterization of Iristatin, a novel immunosuppressive *Ixodes ricinus* tick salivary cystatin
Jan Kotál (České Budějovice/CZ)

T&O-21 Project OSWALD: First data on tick activity in different land cover classes in Germany
Alexander Lindau (Stuttgart/DE)

T&O-23 Ticks (Acari: Ixodidae) infesting cattle in selected districts of Uganda, 2017
Lidia Chitimia-Dobler (Munich, Stuttgart/DE)

T&O-27 Geographical distribution and climate adaptation of the Eurasian hard tick *Haemaphysalis concinna*
Franz Rubel (Vienna/AT)

T&O-31 A proposed biological mechanism of increased aggressiveness of ixodid ticks under high ambient temperatures
Igor Uspensky (Jerusalem/IL)

T&O-33 The problem of *Ixodes* ticks in parks of Moscow City: ways of solution
Yanina Yankovskaya (Moscow/RU)

T&O-35 Comparison of *Ixodes ricinus* populations in adjacent habitats on a pasture-based dairy farm
Annetta Zintl (Dublin/IE)

T&O-37 Screening for novel *I. ricinus* vaccine candidates by probing a novel *I. ricinus* sal ivary gland Yeast Surface Display with sera from forestry workers
Jos Trentelman (Amsterdam/NL)

T&O-39 Identification of novel *Ixodes* vaccine candidates using Yeast Surface Display technology
Jos Trentelman (Amsterdam/NL)

Tick-borne Encephalitis Virus | TBEV-1 – TBEV-17

TBEV-1 Understanding the recent emergence of TBEV in the Netherlands
Julian Bakker (Wageningen/NL)

TBEV-3 Comparative analysis of the tick-borne encephalitis virus load in ticks
Malena Bestehorn (Stuttgart/DE), Giulia Lemhöfer (Munich/DE)

TBEV-5 Isolation of tick-borne encephalitis virus from *Dermacentor reticulatus* and *Ixodes ricinus* in an endemic area in Germany
Martin Pfeffer (Leipzig/DE)

TBEV-7 BONCAT and Click-reaction-on-membrane as a method for identification of differently expressed proteins during TBEV infection of human neural cells
Pavlina Kocova (České Budějovice/CZ)

TBEV-9 Antiviral effect of resveratrol and its derivatives on tick-borne encephalitis virus
Hana Maskova (České Budějovice/CZ)

TBEV-11 Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* and *Dermacentor reticulatus* ticks in Lithuania
Marina Sidorenko (Kaunas/LT)

TBEV-13 Habitat suitability of the TBE virus in Central Europe
Katharina Brugger (Vienna/AT)

TBEV-14 Tick-borne encephalitis associated with consumption of unpasteurised goat milk in Podlaskie Voivodeship in June 2017 – a case series
Joanna Zajkowska (Białystok/PL)

TBEV-15 New areas of TBE incidence in Poland as result of intensification of surveillance
Joanna Zajkowska (Białystok/PL)

TBEV-17 Competence of the vector restricting tick-borne encephalitis virus spread
Katrin Liebig (Hanover/DE)

Please see page 31 for general poster session information.

11:30–13:00 Poster session 2 | Belvedere I–IV & Foyer

Lyme Borreliosis | LB-2 – LB-16

LB-2 Tick-borne pathogens in *Ixodes ricinus* ticks from Ukrainian urban parks
Yuliya Didyk (Bratislava/SK; Kyiv/UA)

LB-4 Genome assembly of the reptile-associated *Borrelia turcica*
Volker Fingerle (Oberschleißheim/DE)

LB-6 Establishment and function of new cytokine elispots for the diagnosis of Lyme disease
Karin Lukas (Regensburg/DE)

LB-8 High species diversity of Lyme disease spirochetes in *Ixodes ariadnae* ticks collected from four *Myotis* species bats in Poland
Justyna Liberska (Poznań/PL)

LB-10 Usefulness of biological samples for *Borrelia burgdorferi* s.l. infection status assessment in avian hosts
Isabel Lopes de Carvalho (Lisboa/PT)

LB-12 Molecular survey of tick-borne encephalitis virus and *Borrelia* diversity in *Ixodes ricinus* ticks from natural habitats in North East Germany
Cristian Raileanu (Greifswald – Insel Riems/DE)

LB-14 Determination of the complement-inhibitory activity of different outer surface proteins of *Borrelia recurrentis*
Florian Röttgerding (Frankfurt a. M./DE)

LB-16 Prioritizing Lyme borreliosis risk areas for forest and nature management based on novel insights in tick ecology.
Mats Van Gestel (Wilrijk, Gontrode/BE)

Tick-borne pathogens | TBP-4 – TBP-36

TBP-4 Emerging pathogens in questing *Ixodes persulcatus* in Karelia (Russia)
Oxana Belova (Moscow/RU)

TBP-6 First report on Q fever cases in patients from Novosibirsk region, Western Siberia, Russia.
Sergey Tkachev (Novosibirsk/RU)

TBP-8 Translational biology of tick-borne diseases: flavivirus infection of tick *ex vivo* organotypic cultures and applications for disease control
Jeffrey M. Grabowski (Hamilton, OH/US)

TBP-10 Molecular detection and characterization of rickettsiae in ticks and mites collected from small rodents in Curonian Spit, Lithuania
Evelina Kaminskienė (Kaunas/LT)

TBP-12 Investigation of the role of tortoises and *Hyalomma aegyptium* ticks in Crimean-Congo hemorrhagic fever epidemiology
Aysen Gargili Keles (Istanbul/TR; Galveston, TX/US)

TBP-14 Development of an in vitro feeding system for ticks as tool to explore the potential transmission routes of Q fever
Sophia Körner (Leipzig, Jena/DE)

TBP-16 Canine breed predisposition in tick-borne diseases
Michael Leschnik (Vienna/AT)

TBP-18 Road-killed vertebrates as sentinel hosts for active surveillance of tick-borne pathogens
Václav Hönig (České Budějovice, Brno/CZ)

TBP-20 Geographically compartmentalized prevalence of *Theileria parva* in the African cape buffalo in Uganda correlates with distribution of the tick vector
Isaiah Obara (Berlin/DE)

TBP-22 *Candidatus* Neoehrlichia mikurensis on the Western Seaboard of Norway. A coldspot within a hotspot.
Benedikte Pedersen (Boe/NO)

TBP-28 Microbiome analysis reveals the presence of *Bartonella* spp. and *Acinetobacter* spp. in deer keds (*Lipoptena cervi*)
Volkhard Kempf (Frankfurt a. M./DE)

TBP-30 Prevalence and molecular characterization of *Anaplasma phagocytophilum* in questing ticks from Galicia (NW Spain)
Susana Remesar (Lugo/ES)

TBP-32 Tick-transmitted diseases in horses in central Germany: Clinical signs, clinicopathological findings, diagnosis, treatment and outcome
Gerald Fritz Schusser (Leipzig/DE)

TBP-34 Screening of ticks collected in Sudan for diverse viruses
Rebecca Surtees (Berlin/DE)

TBP-36 Population genetic analysis and sub-structuring of *Theileria annulata* in Sudan
Diaeldin A. Salih (Khartoum/SD)

Ticks & Others | T&O-2 – T&O-32

T&O-2 The Tick Cell Biobank – new developments and associated research
Lesley Bell-Sakyi (Liverpool/GB)

T&O-4 High numbers of *Hyalomma* ticks in Germany 2018
Lidia Chitimia-Dobler (Munich, Stuttgart/DE)

T&O-8 New finding of *Haemaphysalis concinna* in Western Poland
Dorota Dwużnik (Warsaw/PL)

T&O-10 Factors affecting co-feeding of *Ixodes* spp. ticks on rodent hosts in the Netherlands, an emerging area for TBEV
Helen Esser (Wageningen/NL)

T&O-12 Two year monitoring of tick abundance in the city of Hanover
Andrea Springer (Hanover/DE)

T&O-14 Analysis of skin surface decontamination methods to assess unbiased tick-borne microbiomes
Angeline Hoffmann (Coburg/DE)

T&O-16 Ticks in the close surroundings of football grounds in Germany, a pilot study
Olaf Kahl (Berlin/DE)

T&O-20 Epigenetic modifications of the *Ixodes ricinus* genome
Kateryna Kotsarenko (České Budějovice/CZ)

T&O-22 A comparison between *Ixodes ricinus* nymphs fed in vitro and on calves
Nina Militzer (Berlin/DE)

T&O-26 The effectiveness of the environmental *Metarhizium anisopliae* strain against the local *Ixodes ricinus* population
Dorota Kiewra (Warsaw/PL)

T&O-28 Preliminary results for diurnal questing activity of *Ixodes ricinus* in Leipzig, Germany
Hannah M. Schmuck (Leipzig/DE)

T&O-30 Nonspecific prophylaxis of natural focal diseases caused by ticks *Ixodes* spp. in Russia
Natalia Shashina (Moscow/RU)

T&O-32 Seasonal activity of hard ticks in Vienna
Katharina Brugger (Vienna/AT)

Tick-borne Encephalitis Virus | TBEV-2 – TBEV-16

TBEV-2 The effect of tick saliva on the replication of tick-borne encephalitis virus in different murine macrophage cell lines
Zuzana Berankova (České Budějovice/CZ)

TBEV-4 Biotyping of TBEV-infected IRE/CTVM19 cells
Dmitry Loginov (České Budějovice/CZ)

TBEV-6 Temporal phenology and TBE virus prevalence of *Ixodes* ticks in a German TBE focus over 10 years
Gerhard Dobler (Munich, Stuttgart/DE)

TBEV-8 Detection of temperature-sensitive tick-borne encephalitis virus strains in natural isolates generated from ticks
Giulia Lemhöfer (Munich/DE), Malena Bestehorn (Stuttgart/DE)

TBEV-10 Tick-borne encephalitis virus in cows and unpasteurized cow milk from Norway
Katrine M. Paulsen (Oslo/NO)

TBEV-12 Tick-borne encephalitis virus as one of the components of transmission-blocking anti-tick vaccines
Boris Klempa (Bratislava/SK)

TBEV-16 First phylogenetic analyses of TBE virus detected in Lower Saxony
Mathias Boelke (Hanover/DE)

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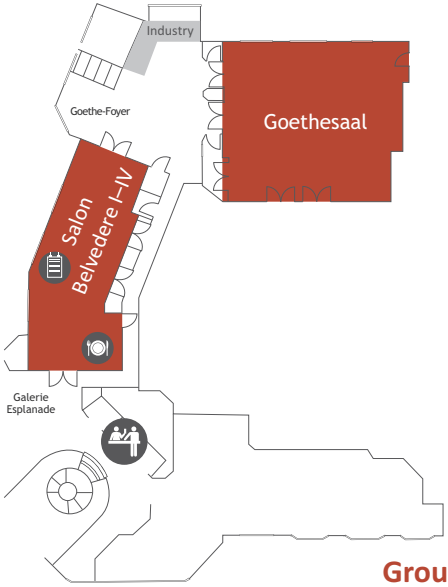
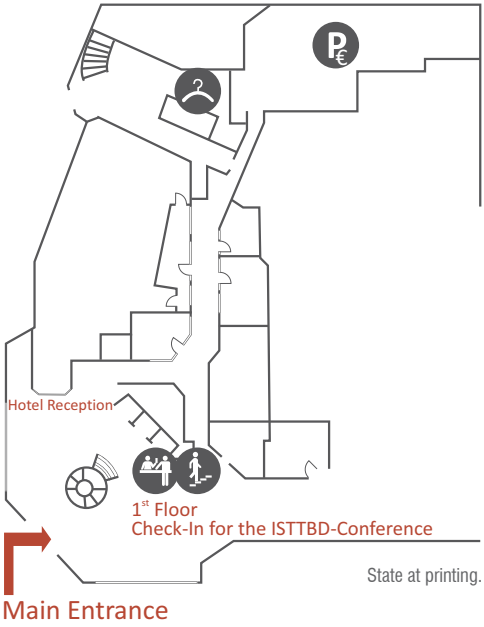


Ground Floor



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Legend

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

Social and cultural programme

Welcome reception I Thursday, 28 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 19:30–21:30
Place Belvedere I–IV & Foyer

Gala dinner I Friday, 29 March

We invite you to join a memorable evening at the "Villa Haar" in Weimar. Celebrate the return of the 13th 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 Michael Nagler will accompany the evening and may even encourage you to dance…

Time 19:00–00:00
Place Villa Haar I Dichterweg 2A | 99425 Weimar, Germany

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.
Taxi's are available upon request through the hotel reception.

Meeting point to walk: 18:45

pictures: Ravpixel.com | Fotolia.com/Harald Wenzel-Orf

General terms and conditions

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

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 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. It costs 2 EUR one way and departs every hour by the hour. The bus takes around 14 to 20 min.

Certification

The conference/congress is certified by the "ATF" (Akademie für tierärztliche Fortbildung der Bundestierärztekammer e. V.) with 16 hours 28–30 March 2019.

Certificate of attendance

Certificates of attendance will be made available on the last day of the conference at the check-in desk.

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.

Online programme

For current detailed information regarding the scientific programme please have a look at our session planer at
https://programm.conventus.de/isttbd2019.

Publication of abstracts

All abstracts will be published in the main programme book as well as will be made available online at www.ittbd-symposium.com.

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 four best poster presentations. Awards will be presented to the winners during the Closing address on Saturday, 30 March from 14:10–15:00.

Submission of a presentation/Technical information

The presentation should be prepared as PDF, MS Office PowerPoint for Windows or key for Macintosh DVD in format 4:3. A presentation notebook with a PDF reader and MS Office PowerPoint 2016 will be provided. The use of personal notebooks is possible upon agreement. However, it may interrupt the flow of the programme in the lecture hall. Please provide an adapter for VGA if necessary. To guarantee a smooth running programme please upload your presentation in due time – at least 2 hours before your presentation is due to start.

Presentation upload

The media check-in for uploading your presentation is located in the back of the plenary Goethesaal. (please follow the signposting). For submission, please use a USB flash drive, CD or DVD disc that is not protected by any software. Professional staff and equipment will be available for you to arrange and preview your presentation. To guarantee a smooth running programme please upload your presentation in due time – at least 2 hours before your presentation is due to start.

Display of name and countdown in projection

Your name will be displayed in the middle upper part of your presentation. Please consider to leave space (ca. 1/10 height at the top of your presentation). A countdown will be displayed on the bottom right corner. The display will be phased out shortly after the beginning of your presentation and phased back in just before the end of your talk.

Time allocation

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

Allotted time is assigned as follows (speaking + discussion time):

1. Keynote	25+5 minutes
2. Short presentations	10 or 15 minutes, with discussion time after each 2–5 presentations

Poster session

Two dedicated poster sessions will be held during the ISTTBD-XIII: on Friday from 10:50–12:20 and on Saturday from 11:30–13:00. Presenting authors of posters with an unequal programme ID, e.g. TBP-1, TBP-3, etc., are requested to be available at their posters to present it to interested ISTTBD-participants on Friday. Presenting authors of posters with an equal programme ID, e.g. TBP-2, are kindly requested to be available for this during the second poster session on Saturday.

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

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

Poster session 1 I Friday, 29 March I 10:50–12:20

All posters of Session 1 have to be hung up by Thursday, 28 March 13:00 and have to be removed by 16:00 on Saturday, 30 March.

Poster session 2 I Saturday, 30 March I 11:30–13:00

All posters of Session 2 have to be hung up by Thursday, 28 March 13:00 and have to be removed by 16:00 on Saturday, 30 March.

Sinnecker Kunz Award

The Sinnecker Kunz Award for early-career researchers (ECRs) will be presented during the 13th International Symposium on Ticks and Tick-borne Diseases (ISTTBD-XIII) in 2019. 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 fourth time that the award will be presented.

ISTTBD-XIII

13th International Symposium on Ticks and Tick-borne Diseases

ABSTRACTS

ORAL PRESENTATIONS

O1
Changing paradigms for tick-borne rickettsioses in North America

C. D. Paddock[1]
[1]Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

During the 21st century, the numbers of reported cases of tick-borne rickettsioses in the United States have followed a consistent upward trend, from approximately 500 cases in 2000 to more than 6,000 cases in 2017. This represents the steepest rise to the highest rate ever recorded. Coupled with remarkable increase in reported cases has been the identification of other, previously unrecognized, tick-borne rickettsial pathogens, including Rickettsia parkeri and Rickettsia 364D. Cases of R. parkeri rickettsiosis, restricted previously to the southeastern and mid-Atlantic states and linked closely to the distribution of Gulf Coast ticks (Amblyomma maculatum), are now recognized in southern Arizona and linked to previously unrecognized populations of Amblyomma triste in riparian canyons of several mountain ranges in this region. Rickettsia parkeri-infected specimens of A. triste are also now recognized in northern Mexico. Although R. parkeri and Rickettsia 364D rickettsioses are clinically, epidemiologically, and ecologically distinct from the prototypical spotted fever rickettsiosis, RocLky Mountain spotted fever (RMSF), cases of R. parkeri and 364D rickettsiosis have undoubtedly been embedded among national surveillance data for RMSF for decades. Since 2003, epidemic levels of RMSF have emerged in the southwestern United States, as well as many regions of Mexico, particularly in the states of Baja California and Sonora, where case fatality rates range from approximately 30-40%. These epidemiology and ecology of these outbreaks are distinct from classical RMSF and associated specifically with large populations of free-roaming dogs that perpetuate enormous numbers of Rhipicephalus sanguineus sensu lato ticks in urban or community settings. Collectively, these observations demonstrate several themes: (1) the scope and magnitude of tick-borne rickettsioses are constantly evolving and expanding; (2) changes in the distribution and determinants of these diseases may occur over relatively brief intervals of time and space; and (3) the epidemiology of historically recognized tick-borne rickettsioses may evolve alongside the discovery of newly characterized pathogens. This presentation examines the growing list of tick-borne rickettsioses and briefly considers certain aspects of the dynamic and multifaceted natural histories of these diseases in the United States and Mexico.

O2
Tick-borne encephalitis virus of Western and Eastern Siberia – genetic diversity, geographical distribution and evolution

S. Tkachev[1], I. Babkin[1], G. Chicherina[2], I. Kozlova[3], M. Verkhozina[4], T. Demina[5], O. Lisak[3],E. Doroshchenko[3] Y. Dzhioev[6], O. Suntsova[3], J. Savinova[3], A. Paramonov[3], A. Tikunov[1], V. Zlobin[6], D. Ruzek[7,8], N. Tikunova[1]
[1]Institute of Chemical Biology and Fundamental Medicine SB RAS, Laboratory of Molecular Microbiology, Novosibirsk, Russian Federation
[2]Institute of Systematics and Ecology of Animals SB RAS, Novosibirsk, Russian Federation
[3]Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russian Federation
[4]Center for Hygiene and Epidemiology in the Irkutsk Region, Irkutsk, Russian Federation
[5]Irkutsk State Agrarian University by A.A. Ezhevsky, Irkutsk, Russian Federation
[6]Irkutsk State Medical University of the Ministry of Health of Russia, Irkutsk, Russian Federation
[7]Veterinary Research Institute, Brno, Czech Republic
[8]Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic

Introduction: Tick-borne encephalitis virus (TBEV) is currently divided into three subtypes - the European (TBEV-Eu), the Far-Eastern (TBEV-FE), and the Siberian (TBEV-Sib). Within each subtype, genetic lineages have been described. Although attempts to study the variability of TBEV in Western and Eastern Siberia have been made previously, the detailed analysis based on appeared new data hasn"t been performed yet.
Objectives: The aim of our study was the detailed analysis of TBEV genetic diversity, geographic distribution and divergence time of different subtypes and genetic lineages of TBEV from Western and Eastern Siberia.
Materials & methods: Molecular-genetic analysis was based on E gene fragments, complete genome sequences, and all currently available data in the GenBank database.
Results: TBEV-Sib was shown to dominate in Siberia region; TBEV-FE and TBEV-Eu can also be found. The study of TBEV-Eu strains isolated in Western and Eastern Siberia identified two genetic lineages differed genetically from each other. It was shown that within TBEV-Sib, Zausaev and Vasilchenko lineages are distributed both in Western and Eastern Siberia whereas the Baltic lineage and a novel Obskaya lineage are presented only in Western Siberia; the Obskaya lineage diverged from the common ancestor the earliest, the Baltic lineage was separated later, and other lineages diverged the most recently. Also, a group of TBEV strains with unique genetic structure isolated in Eastern Siberia was identified. These TBEV variants formed an independent cluster on dendrograms, had no high homology with any strains of three main genotypes and were proposed to be new, Baikalian TBEV subtype.
This study was supported by the Program of Fundamental Scientific Research of the State Academies of Sciences (project No. 55.1.1), research work № *01201282421 (*0542-2014-0006), and the Russian Foundation for Basic Research and the Government of the Novosibirsk Region (research project No. 18-41-000001).

O3

Properties of the tick-borne encephalitis virus population during acute and persistent infection of ixodid ticks

O. Belova[1,2], A. Litov[1], I. Kholodilov[1], L. Kozlovskaya[1,3], L. Romanova[1,3], G. Karganova[1,4]
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[2]Martsinovsky Institute of Medical Parasitology, Tropical and Vector Borne Diseases, Sechenov University, Moscow, Russian Federation
[3]Institute for Translational Medicine and Biotechnology, Sechenov First Moscow State Medical University, Moscow, Russian Federation
[4]Lomonosov MSU, Faculty of Biology, Moscow, Russian Federation

Tick-borne encephalitis virus (TBEV) is the causative agent of tick-borne encephalitis (TBE) – a tick-borne zoonotic neuroinfection. In recent years, changes in the eco-epidemiology of TBEV due to changes in distribution of ixodid ticks have been observed. These changes could result in a shift of the main tick vector species, which in turn may lead to changes in properties of the virus.
In the present study we evaluated the selective pressure on the TBEV population during acute and persistent infection of various species of ixodid ticks.
To adapt TBE strain of Siberian subtype to acute infection in ticks of different species, from 2 to 7 parenteral passages in *Ixodes ricinus*, *I. persulcatus*, *Dermacentor silvarum*, *D. reticulatus* ticks from laboratory cultures were carried out. To obtain tick-adapted variants with long persistence in their bodies, we infected percoxally *I. ricinus*, *I. persulcatus* and *D. reticulatus* ticks with TBEV strains of European and Siberian subtypes. Infected ticks were kept at room temperature for up to 5 months and at different time points we collected between 2 and 6 ticks for further analysis. For all TBEV variants obtained in experiments the virus titre and plaque phenotype were determined, and for some variants neuroinvasiveness for laboratory mice was defined and the nucleotide sequence of the virus genome fragment encoding E protein was analysed.
We showed that during experimental infection TBEV effectively replicated and formed persistent infection in ticks. Adaptation of TBEV to acute infection in ticks of different species led to the increased heterogeneity of the viral population and selection of TBEV variants with reduced neuroinvasiveness for laboratory mice and with mutations in the E protein that increased local positive charge of the virion. During persistent TBEV infection in ticks of different species, an increase in the heterogeneity of the viral population was observed without rapid selection of such mutants.

O5
Temporal and spatial epidemiology of human tick-borne encephalitis in Germany from 2016 to 2018

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[1]Bundeswehr Institute of Microbiology, Virology and Rickettsiology, Munich, Germany
[2]University Hohenheim, Dept. of Parasitology, Stuttgart, Germany

Introduction: Tick-borne encephalitis (TBE) is the most important tick-transmitted viral disease in Europe and Asia. The ecological, climatic and social factors, which interfere with the epidemiology of TBE are still poorly understood.
Objectives: Aim of the current study was to analyse the spatial and temporal occurance of human TBE cases in Germany in 2018 and to compare the incidence data of the years 2016 and 2017. With this comparison a better understanding of the spatial and temporal occurance of human TBE cases under different conditions of 2016 to 2018 are generated and possible factors for the epidemiology of TBE be identified.
Methods: Registered human TBE cases at the Robert-Koch-Institut (available at SurvStat) of the years 2016 to 2018 were analysed according to their spatial and temporal occurrance and compared on district and state level. Furthermore, the weekly notifications of human TBE cases for the three years were compared to each other and to the average values of 2001 to 2015.
Results: 2016 was a year with an average number of human TBE cases and temporal and spatial distribution. 2017 was the year with the second highest number of registered human TBE cases after 2006. The main reason was a high peak of registered cases in weeks 25 and 26 and an autumn peak in the State of Bavaria. In 2018 the highest number of TBE cases ever in Germany was registered. The temporal analysis showed a high number above average of recorded cases during the weeks 19 to 27, which is exactly the time of hot and try weather in 2018. The spatial analysis of the three years shows marked increases of TBE incidence rates in the south and decreases in the north of the states of Bavaria and Baden-Württemberg.
Conclusion: The incidence rates of human TBE in 2016 to 2018 were depending on short-term weather conditions but also on spatial changes in larger regions within the endemic belt of TBE in Germany.

O6
Phylogeny of TBE virus in Austria and Central Europe

M. Bestehorn[1], L. Chitimia-Dobler[1,2], K. Stiasny[3], G. Dobler[1,2], F. Heinz[3]
[1]University of Hohenheim, Parasitology, Stuttgart, Germany
[2]Bundeswehr Institute of Microbiology, Munich, Germany
[3]Medical University of Vienna, Institute of Virology, Vienna, Germany

Introduction: Tick-borne encephalitis virus (TBEV) is the most important tick-borne virus in Europe and Asia. While the number of TBE cases in the eastern parts of Austria is declining there are recently reported cases in Tirol and Vorarlberg, two federal states which not so long ago were thought of to be TBE free (Heinz et al. 2015).

Methods: For a better understanding of the spread of TBEV in Austria and Central Europe, ticks were sampled and screened for TBEV in different federal states of Austria. The viruses from the positive samples were isolated and whole genome sequences were generated. Further we were able to sequence historic TBEV strains which were kindly provided by Prof. Heinz, Vienna. A phylo-geographic analysis was conducted forming a relation of genetics and geographic places of occurrence.
Results: Our findings indicate that TBEV was introduced several times to different areas of Austria. The resulting data show that the isolates from the 1970s and 1980s cluster in phylogenetic arbitrary genotypes with viruses from Germany and the Czech Republic but also with strains recently isolated in Austria. These results show that some of the recently emerged TBE endemic areas in Austria are not formed by a continuous spread of TBEV strains from existing natural foci along natural landscape aisles (river valleys of Ziller and Inn), but seem to be recently introduced from other regions in Central Europe.
Discussion: The dynamics of TBEV appearance, disappearance and spread seems to be much more complicated than thought before. The mode of introduction and spread are so far unclear; however our recent phylogenetic data from Germany suggest that migratory birds, terrestrial animals and anthropogenic effects may play a role in the recent spread of TBEV in Europe and its emergence in new regions.

O7
Detection of tick-borne encephalitis virus antibodies in sera of sheep and goats in Mecklenburg-Western Pomerania (North-Eastern Germany)

S. Frimmel[1], M. Löbermann[1], F. Feldhusen[2], M. Seelmann[2], K. Stiasny[3], J. Süss[4], E. C. Reisinger[1]
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[2]State Institute for Agriculture, Food Safety and Fisheries Mecklenburg-Western Pomerania, Rostock, Germany
[3]Medical University of Vienna, Center for Virology, Vienna, Austria
[4]Brehm-Gedenkstätte Renthendorf, Renthendorf, Germany

Introduction: In Mecklenburg-Western Pomerania, a federal state in the north east of Germany and one of the most popular holiday regions in Germany no district has ever been declared as a risk area of tick-borne encephalitis (TBE) infection. Autochthonous cases, along with TBEV-RNA detection in ticks, have shown activity of the virus in natural foci in the past. Sentinel animals like sheep and goat are of special interest in regions of low prevalence of TBE to assess the risk of TBE infection.
Methods: A total of 479 sera from livestock from 18 farms in different regions of Mecklenburg-Western Pomerania were investigated, 375 from sheep including 65 ewes and 6 rams and 104 sera from goats, including 23 breeding goats. All sera were obtained for routine examination between 2014 and 2017. The samples were tested using the “Immunozym FSME IgG All Species ELISA kit®” and virus neutralization test (NT) was performed as control for all positive (>126 VIEU) or borderline (63 - 126 VIEU) sera. Seropositivity in NT was defined as a titer of ≥1:10.
Results: Eleven of 479 sera tested borderline for anti-TBEV-IgG with ELISA (2.3%). Nine of 375 serum samples from sheep (2.4%), and two of 104 samples from goats (1.9%) were ELISA borderline. Two samples of sheep sera tested positive using NT. One sample from the administrative district

Mecklenburgische Seenplatte from the year 2014 and one sample from the district Vorpommern-Greifswald from the year 2016 were tested positive by NT.
Conclusions: To the best of our knowledge, the two positive sera constitute the first detection of TBEV-antibodies in sera of livestock in Mecklenburg-Western Pomerania since the sixties of the last century. The presence of antibodies against TBEV in livestock animals underlines that the serological examination of domestic animals like sheep and goat is a useful tool in defining areas of possible TBEV infection in regions of low endemicity.

O8
Ixodid ticks and tick-borne encephalitis virus in the Republic of Tuva

I. Kholodilov[1], O. Belova[1,2], L. Burenkova[1], Y. Korotkov[1], L. Romanova[1,3], L. Morozova[4], L. Gmyl[1]
I. Belyaletdinova[1], A. Chumakov[5], N. Chumakova[5], O. Dargyn[6], N. Galatsevich[5], A. P. Gmyl[1], N. Oorzhak[7]
A. E. Polienko[1], A. Saryglar[7], V. Volok[1], A. Yakovlev[1], G. Karganova[1]
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The most significant processes of arbovirus evolution can be expected at the boundaries of the viruses" areas and ticks" cohabitant areas, where the probability of the appearance of new virus variants is high due to the change of the main vectors and/or hosts. One of the most interesting regions is the Republic of Tuva (Tyva). Most of the territory is occupied by mountain ranges and a few intermountain basins that allow us to study the area of vectors and viruses at different altitudes, in geographically isolated areas, and in various landscape areas. This work is devoted to the study of ticks and the tick-borne encephalitis virus (TBEV) areas in Tyva. From 2008 to 2017, we conducted six expeditions and collected by flagging and from domestic animals 2761 adult ticks, 15 nymphs. All ticks were examined for the presence of TBEV. The distribution of ticks in Tyva is spotty. In the forest zone *Ixodes persulcatus* is the most important epidemiologically significant TBEV vector. In the forest-steppe and steppe zones, on the plains and slopes of the mountains, all three species of ticks are found. *D. silvarum* disappears after 1300 m above sea level (a.s.l.). Above that altitude only *D. nuttalli* (the highest point is 2016 m a.s.l.) and *I. persulcatus* (the highest point is 1538.7 m a.s.l.) are found. We isolated 14 strains of TBEV, 12 strains from *I. persulcatus*, 1–*D. nuttalli,* 1–*D. silvarum*. All strains refer to the Siberian subtype and form one monophyletic group. TBEV was found at all altitudes. The highest points where TBEV was identified were 1538.7 m a.s.l. for *I. persulcatus*, 1520 m a.s.l. for *D. nuttalli*, and 1031 m a.s.l. for *D. silvarum*. There are no significant

differences between the infection rate of *Dermacentor* ticks and *I. persulcatus* in places where one species is represented or in cohabitation zone.
The distribution of tick species is confined to a specific landscape. TBEV of the Siberian genotype circulates on the territory of Tyva in all tick species.

O9

Canine anti-α-Gal antibodies – Potential role in red meat allergy and protection against tick-borne pathogens

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Specific IgE antibodies (Abs) against the carbohydrate galactose-α-1,3-galactose (α-Gal) has recently been linked to delayed anaphylactic reactions to red meat in human patients previously exposed to tick bites. In dogs, α-Gal is a self-antigen and their immune system is not expected to naturally produce Abs toward the glycan molecule. However, results of this preliminary study demonstrated the specific immune response to α-Gal in dogs and suggested its relation to ticks, red meat allergy and protection against tick-borne pathogens (TBPs). Serum samples from 86 dogs from Austria were tested for Abs (IgG, IgM, IgE) against α-Gal conjugate and *Ixodes ricinus* and *I. scapularis* salivary gland proteins (SGP) by indirect ELISA. Dog exposure to TBPs was assessed by molecular and serological methods. In addition, serum from a 3-year-old dog, displaying delayed allergic reaction after red meat consumption, was also included in this study. Serum IgG and IgM against α-Gal were detected in 86 and 85 animals, respectively. Half of the dogs, including the one allergic to red meat, had detectable IgE to α-Gal. A strong correlation was observed between IgM and IgE Ab levels to α-Gal and to *I. ricinus* SGP. Interestingly, the dog sera also recognized the epitopes of *I. scapularis*. Significantly higher levels of anti-α-Gal IgM were recorded in dogs negative to *Anaplasma phagocytophilum* infection compared to the exposed ones.
Our results demonstrate the occurrence of canine Abs against α-Gal for the first time, and suggests that a tick bite can sensitize dogs to α-Gal and potentially trigger delayed allergic reactions to red meat following the induction of specific IgE. The results also suggest the protective role of anti-α-Gal Abs against TBPs. Our findings open a completely new scientific perspective, which may contribute to a better understanding of the mechanisms involved in the pathogenesis of this unique allergy in the future, and may help to prevent canine vector-borne diseases.

O10

Feeding-induced salivary gland genes of *Ixodes ricinus* ticks as targets for anti-tick vaccines

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Ticks, as vectors, are only second to mosquitoes in transmitting pathogens that cause diseases with substantial health and economic burden. A lack of commercial vaccine available for human use clearly underscores the urgent need for development of new vaccine candidates which are able to confer comprehensive protection against multiple tick-borne diseases. As part of the ANTIDoTE project, we have identified several vaccine candidates in salivary glands of independent *Ixodes ricinus* populations by implementing a transcriptomic approach that combines RNA and **m**assive **a**nalysis of **c**DNA **e**nds (MACE). The salivary gland genes, upregulated at different time points, of both infected (with *Borrelia afzelii*, Tick-borne encephalitis virus or *Babesia microti*) and uninfected ticks were evaluated. Due to significant biological variation, observed among different tick populations, we selected genes that were robustly upregulated only at 24 hours post-feeding in uninfected ticks in four independent MACE datasets. The selection was based on two arbitrary pre-defined criteria in all 4 datasets: i) average number of normalized reads at 24 hours >100 (used for ranking); ii) log2 fold-change of >2 at 24 hours vs 0 hour, of feeding. Based on this analysis, a total number of 124 transcripts/genes, commonly present in all datasets, were identified and the top 25 were selected for further analysis and validation. *In silico* gene expression was checked in all the datasets. Seventeen transcripts were biologically validated with quantitative real time PCR (qRT-PCR) in three independent cDNA pools derived from salivary glands of uninfected *I. ricinus* nymphs. All contigs tested, with the exception of no_Hit_Assembly_70916 and GXP_Contig_24224, demonstrated a robust up-regulation at 24 hours and/or at fully-fed conditions in MACE datasets and qRT-PCR, respectively. In the near future, double stranded RNA (dsRNA) will be synthesized for the corresponding contigs to perform RNAi (gene silencing/knockdown) using a nano-injector to substantiate their role in the tick feeding process and we have initiated the production recombinant proteins of a subset of these targets to test them as anti-tick vaccine candidates. In theory, these transcripts/genes might be indispensable for successful tick feeding and targeting these could impair tick feeding as well as pathogen transmission. Therefore, these genes and the corresponding proteins might be ideal anti-tick vaccines that could, in principle, provide protection against a wide range of tick-borne pathogens.

011

Interpopulation differences in behavior of ixodid ticks

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Abiotic and biotic factors of the environment influence on all members of the parasitic system "virus-tick-host" and, in particular, determine the characteristics of the life cycle, physiology and behavior of ixodid ticks, which causes the appearance of interpopulation differences of ticks of the same species. The aim of this work was to study the interpopulation differences in behavior and biology of ixodid ticks. For that purpose, in 2017-2018 we collected ticks in 5 regions of the Russian Federation, where habitat of ticks of one species differed: *Ixodes ricinus* – Kaliningrad and Voronezh regions*, I. persulcatus* – Republic of Tuva and Karelia, and *Dermacentor reticulatus* – Kaluga and Voronezh regions.

To compare ticks populations of one species, the following experiments were conducted:

1. Evaluation of the reaction of ticks to ethanolic extracts of plants collected in different biotopes with high number of ticks. As a result, differences in the reaction of different populations of ixodid ticks to some plant extracts were revealed.
2. Study of ticks" activity and tolerance to the repellent DEET (diethyltoluamide). To assess the activity of ticks, we analysed the height of ticks" trajectory, their path and speed over 3 minutes of observation on a tape impregnated with increasing concentrations of DEET. In our experiments, *I. ricinus* ticks from Voronezh region, *D. reticulatus* ticks from Kaluga region and *I. persulcatus* ticks from the Republic of Karelia showed higher activity and were more tolerant to the DEET, than ticks of the same species from Kaliningrad region, Voronezh region and Republic of Tuva, respectively.
3. Study of the resistance of ticks of the same species from different climatic regions to elevated temperatures. The experiments were carried out in a cold cabinet (4°C-17°C) and in a climatic chamber (22°C-30°C) according to Gilbert L. et al. (2014). We established significant difference in the reaction of different populations of ticks of the same species to the temperature increase. Populations of ticks collected from warm regions were the most resistant to high temperatures (above 26°C) and remained active longer.

As a result of the experiments, we obtained data indicating interpopulation differences of ticks of the same species collected in different regions.
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O12
Dynamics of the tick pathobiome

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Ixodes ricinus is the predominant tick species in western Europe and is recognised as the primary vector of pathogens in humans. High throughput sequencing approaches showed that they also carry other micro-organisms. The composition and diversity of this microbiota may have an impact on pathogen presence. These findings led to elaborate the concept of pathobiome which considers a pathogen within its abiotic and biotic environments, including other micro-organisms of this environment. Few studies have currently characterized the structure and the temporal dynamics of the tick pathobiome and data on potential co-occurences between pathogens and the tick microbiota remain scarce.

In this context, we studied the temporal dynamics of *I. ricinus* micro-organisms (pathogen and microbiota). Over three consecutive years, ticks were collected monthly in a peri-urban forest in the south of Paris (France). About 1000 nymphs were collected and individually analysed for the presence of 30 pathogens using a high throughput screening method (microfluidic PCR). In parallel, the structure and composition of tick microbiota were identified by 16s rRNA gene sequencing. Pathogens belonging to four pathogenic genera (*Anaplasma* spp, *Rickettsia* spp, *Borrelia* spp and *Babesia* spp) were detected in 16% of the collected nymphs. Statistical analysis allowed to observe significant differences of pathogen prevalences according to season and year. The analysis of the tick microbiota showed that the five most abundant sequences belonged to the genera *Arsenophonus*, Candidatus *Midichloria*, *Rickettsia*, *Wolbachia* and *Spiroplasma*, all corresponding to maternally inherited bacteria in ticks. Otherwise, statistical analysis allowed to show significant differences in the tick microbiota composition, between ticks collected at the beginning and at the end of the year. We observed that these differences were highly correlated with some abiotic factors and the presence of some pathogens.

O13
The *Ixodes ricinus* salivary gland proteome during feeding and *Borrelia* infection

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Introduction: *Borrelia burgdorferi* sensu lato, the causative agent of Lyme borreliosis, is transmitted by *Ixodes* ticks within 24-48 hours of attachment. Salivary proteins facilitate tick feeding and the transmission of *B. burgdorferi*. In turn, spirochetes induce the expression of tick salivary gland proteins, which facilitate their transmission to the host.
Objectives: To use comparative proteomics in ticks under different feeding and infection conditions to identify salivary gland proteins induced during feeding and in response to spirochetal infection as possible vaccine candidates.
Materials & methods: Salivary glands of *B. afzelii* strain CB43 infected and uninfected *Ixodes ricinus* nymphs were dissected from unfed, 24 hour fed and fully fed ticks and total protein was isolated. Label-free Quantitative Proteomics and Progenesis QI software was used to identify and compare tick salivary gland proteins expressed during feeding and in response to *Borrelia* infection. Experiments were performed in quadruplicates.
Results: We identified 922 *I. ricinus* proteins of which 921 are also present in the only *I. ricinus* proteomics analysis published to date or in an *I. ricinus* RNA sequencing database previously generated as part of another study. From the 922 identified proteins, 68 were upregulated upon 24-hour feeding and *Borrelia* infection. We generated 7 recombinant proteins in either *E. coli* or *Drosophila* and have validated 3 so far by Western blot, which have been used in vaccination studies. Further validation and vaccination studies are ongoing.
Conclusion: Quantitative proteomics identified differential protein expression in *I. ricinus* salivary glands in response to *Borrelia* infection and different feeding conditions. These results will provide insights into tick feeding and *Borrelia* transmission and might reveal novel vaccine candidates for an anti-tick vaccine.

O14
The factors that shape vector aggregation on small mammal hosts

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Lyme disease is the most prevalent tick-borne disease in Europe and the United States. However, researchers rarely examine host-mediated vector distributions that shape Lyme disease dynamics. In the western U.S., the blacklegged tick, *Ixodes pacificus* is the main vector for *Borrelia burgdorferi;* the spirochete causing Lyme disease. Juvenile stages of *I. pacificus* feed on reservoir hosts such as deer mice (*Peromyscus spp.*) and dusky-footed woodrats (*Neotoma fuscipes*). Small mammals – the key reservoirs for *Borrelia spp.* - can vary greatly in abundance depending on population dynamics

and habitat context. Tick burdens on small mammals is an important parameter in estimating and predicting Lyme disease risk as transmission can be amplified by larger tick aggregation on certain hosts. This study sought to understand how host community composition, diversity and tick abundance affected *I. pacificus* larval burdens on reservoir competent hosts in a Lyme endemic region. A total of 205 small mammals were captured, predominately *N. fuscipes* (dusky-footed woodrat) and three different *Peromyscus* species (deer mouse) and 325 *I. pacificus* larval ticks were collected from these hosts. Average *I. pacificus* larval burdens were significantly higher on *N. fuscipes* compared to *Peromyscus spp*.. Generalized linear models found that mean larval *I. pacificus* burdens on *N. fuscipes* increase significantly with small mammal diversity and questing *I. pacificus* densities but decrease as predator diversity increases. Larval burdens on *Peromyscus spp.* increase significantly with an increase in small mammal diversity as well as *Peromyscus spp*. abundance but decrease as predator diversity increases. Thus, this study illustrates how host-mediated dynamics can result in altered distribution of pathogen vectors. These results have important implications for understanding tick distributions on hosts and are important for predicting Lyme disease transmission, prevalence, and ultimately human health.

O15

Does tick immune system fight with tick-transmitted pathogens?

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Ixodes ricinus is the most common tick in Europe. It transmits important human diseases like Lyme borreliosis, Tick-borne encephalitis, and Babesiosis. The tick innate immune system has capabilities to distinguish between self and non-self to fight the pathogens and possibly reduce or block their transmission. Here, we report an overview of the tick immune system and focus on characterization of fibrinogen-related proteins (FRePs) and thioester-containing proteins (TEPs). These two groups of the hemolymph proteins are supposed to work together to trigger phagocytosis of pathogens by tick hemocytes or cause direct lysis of the pathogens. Further, we present several laboratory infection models able to study pathogens acquisition and transmission. The tick immune molecules and transmission systems are compared with the laboratory model of malaria mosquito *Anopheles gambiae*. Identification of important tick molecules should help us to better understand and focus our fight against ticks and pathogens.

O16
Rhipicephalus rossicus – overview of a neglected tick knocking at the door of Europe

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Question: Ticks are one of the most medically important groups of arthropods. They are vectors of several important diseases with high associated health-care costs and impacts. Most of tick-borne diseases show an emerging pattern in Europe.
Methods: This could be explained by two main hypotheses: (1) changes of geographical distribution of tick ranges and (2) increased contact of ticks with the human population. Rhipicephalus rossicus is among the tick species with recent range expansion in Eastern Europe. This understudied species raises many challenges among researchers. Its distribution ranges primarily in Romania, Ukraine and other former Russian countries in the steppe habitat.
Results: It has been shown that this species is parasitizing small mammals and ruminants, being the proven vector for Crimean-Congo Hemoragic Fever, Q-fever, tularemia and West Nile virus. However, recent studies highlighted its occurrence in SE Romania, with a dominant presence on dogs. This finding is particularly interesting because dogs commonly share a high number of tick species with different vectorial roles. Among these, in the studied region, *R. sanguineus*, was commonly found on dogs. Despite this tick species is related genetically and morphologically to *R. rossicus*, it is reported to be competent vector for completely different pathogens such as several species of Rickettsia (spotted fever group), *Anaplasma* spp., or *Cercopithifilaria* nematodes, none yet known to be vectored by *R. rossicus*.
Conclusions: The close similarity of these two tick species, their sympatric co-occurrence in Romania and the same host range may provide a unique situation for exploring the pathogen bridging. Moreover, the distribution of *R. rossicus*is is not well tracked in countries bordering the eastern part of Romania. The potential expansion of *R. rossicus* in other steppe areas of eastern Europe will probably find unprepared veterinarians and authorities, posing new risks for animal and public health.

O17
Identification and characterization of catalase from the hard tick *Haemaphysalis longicornis* and evaluation of its *in vitro* antioxidant activity

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Introduction: Ticks are known to lack heme metabolism pathways because several heme synthetases and a heme oxygenase are defective at genomic levels. Therefore, ticks put the host-derived heme into hemosomes of the midgut without digestion. These facts indicate that it might be difficult to produce the heme-containing proteins, such as a catalase, which is one of the hydrogen peroxide (H_2O_2)-scavenger. However, recent reports showed that the catalase gene and protein are present

in the ticks and help transmission of a pathogen from adult female ticks to their eggs.
Objectives: We focused on tick catalase and evaluated its antioxidant activity on scavenging H_2O_2 using a recombinant protein expressed by *Escherichia coli*.
Materials & methods: A catalase gene (*HlCAT*) was identified from *Haemaphysalis longicornis* and its biological function was evaluated. First, *in silico* analyses of the *HlCAT* nucleotide and amino acid sequences were performed. Then, a recombinant HlCAT (rHlCAT) was prepared by *E. coli* expression system. Finally, an antioxidant activity of the expressed rHlCAT protein was examined using a mixed oxidation (MFO) assay.
Results: *HlCAT* obtained from the cDNA library of *H. longicornis* had 1,500 bp open reading frame, encoding 499 amino acids with a predicted molecular mass of approximately 57.1 kDa and pI of 8.13. Signal peptides and N-glycosylation sites were not predicted. HlCAT had a catalase domain at amino acid positions 23 - 407. When the antioxidant activity of the rHlCAT protein was examined using the MFO assay, the protein showed antioxidant activity.
Conclusion: These results suggest that the catalase gene is present in ticks and the recombinant catalase has antioxidant activity *in vitro*. However, there is no report about the presence of catalase protein in ticks, thus, further studies will be needed to understand its biological functions in ticks.

O18
Comparison of tick diversity between adult and juvenile red foxes

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Introduction: Red fox inhabits different types of environment, from forests and meadows to suburban and highly urbanized areas. Since foxes explore large areas in search of food, they are prone to becoming infested with a wide variety of tick species.
Objectives: We determined tick fauna on red foxes in Poland and compared the diversity of tick species collected from adult individuals acquired in winter/fall season and those found on pups trapped during fox breeding season (May-June). The phenomenon of subcutaneous tick occurrence was also investigated.
Materials & methods: 366 fox carcasses were obtained from legal hunting from three regions of Poland. To detect parasites in subcutaneous tissue foxes were skinned. Molecular typing was conducted to determine tick species involved in this phenomenon. Twenty five fox pups were live-trapped in vicinity of fox burrows. Ticks were collected from pups under pharmacological anesthesia.

Results: 5 tick species were found on examined foxes. Prevalence and main abundance of ticks infestation between adult foxes acquired in winter/fall season and cubs was extremely different. Much higher tick infestation was found on juvenile foxes (60%) than on adults (15.6%). The most common tick species on adult foxes were *D. reticulatus* (6.6%), *Ixodes canisuga* (6.0%) and *I. ricinus* (5.2%). Rare species was *I. hexagonus* (3.6%). On pups the highest prevalence (56%) was of *I. ricinus*, then *I. canisuga* (16%) and *I. hexagonus* (5 nymphs on one cub). Only one female of I. kaiseri was found on an young fox. Infestation with subcutaneous tick was recorded in 38% of autopsied foxes and all genotyped specimens (n=21) were identified as *I. ricinus*.
Conclusion: Red fox constitutes a host of many tick species of ecological significance and plays an important role in spreading parasites amongst various habitats.
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O19
The distribution limit of *Ixodes ricinus* in north-western Europe

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Introduction: In 1943, the northern distribution limit of *Ixodes ricinus* was considered to be at Brønnøysund (65°28 N, 12°12" E). Now there is increased focus on the possible expansion of this vector"s distribution in Norway.
Objectives: The project *Ticks in northern Norway* started in 2009 with the intention of studying the tick"s northern distribution limit in northern Norway.
Materials & methods: Ticks were collected by flagging/dragging, from live-trapped rodents and from pets from multiple sites in northern Norway and their presence correlated with the vegetation growing season. Ticks were analysed for *Borrelia burgdorferi* sensu lato (s.l.) by PCR.
Results: During the summer seasons 2010 - 2018, flagging/dragging was performed on 167 occasions in 109 different locations in 31 municipalities in the counties of Troms and Nordland in northern Norway and in Trøndelag County. - In seven (6%) examined locations, larvae were collected and thereby fulfil the criteria proposed at the Consensus Conference on Lyme Disease in 1991 for permanent *I. ricinus* populations. The northernmost such site was at Nordøyvågen on the north coast of the island of Dønna (66°12"N, 12°35"E), where all three tick stages were found in two successive years. Additionally, few ticks were collected at pet clinics in Troms County and in the archipelagos of

Lofoten and Vesterålen. *I. ricinus* larvae were not found on rodents north of Dønna. - The prevalence of *B. burgdorferi* s.l. spp. in the 557 ticks collected in 2011-16 was low at the distribution limit but increased southwards within the study area.
Conclusion: All the tick stages (larva, nymph, female, male) were demonstrated by flagging in successive years at Nordøyvågen, on the island of Dønna. This constitutes evidence of a permanent *I. ricinus* population, which may be the northernmost reported so far. Our results make it possible to estimate the length of the growing season that defines the geographical limits of *I. ricinus* establishment.

O20
Isolation and propagation in tick cell lines of *Spiroplasma* spp. from European *Ixodes* and *Dermacentor* ticks

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Introduction: While several molecular studies have indicated the presence of *Spiroplasma* in European ixodid ticks, very little is known about the interactions between these ticks and the *Spiroplasma* species that they harbour.
Objectives: Isolation and *in vitro* propagation in tick cell lines would facilitate research on *Spiroplasma* endosymbionts of ticks.
Materials & methods: Adult ticks collected from sites in Slovakia, Poland, The Netherlands and Spain provided either internal organs (from unfed or partially-fed males and females) for inoculation into a panel of tick cell lines or eggs (laid by engorged females) for generation of primary cell cultures. Molecular characterisation of isolated *Spiroplasma* (PCRs targeting 16S-23S rRNA intergenic transcribed spacer, RNA polymerase beta subunit and 16S rRNA) was used to determine phylogeny.
Results: Host species-specific *Spiroplasma* strains were isolated from *Ixodes ricinus* (n=5), *Dermacentor reticulatus* (n=4) and *Dermacentor marginatus* (n=1).
Conclusion: Availability of three genetically different *Spiroplasma* strains harboured by three common European tick species provides opportunities for further comparative study of these enigmatic microorganisms.

O21

Rhipicephalus sanguineus group – an update of the taxonomic status and its epidemiological implication

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The *Rhipicephalus sanguineus* group includes several species such as *Rhipicephalus sanguineus* s.s., *R. sulcatus*, *R. rossicus*, *R. schulzei*, *R. pumilio, R. pusillus, R. turanicus, R. leporis, R. guilhoni*, *R. moucheti,* and *R. camicasi*. *Rhipicephalus sanguineus* s.s. is, from a public health and economic perspective, the most important species of this species group. In spite of the veterinary, medical and economic relevance of *R. sanguineus* s.s., its name has often been applied to any population of *Rhipicephalus* ticks of the *R. sanguineus* group associated worldwide with dogs. This was often done without following any strict formal, biological, morphological or molecular criteria. But recently, a neotype of *R. sanguineus* s.s. was designated, all parasitic stages were morphologically described, and DNA sequences of different molecular markers are now available. This re-definition of *R. sanguineus* s.s. as a biological entity constitutes a benchmark against which the taxonomic and ecological diversity represented by the ticks currently assigned to this name could be compared. This would also allow a more acute assessment of epidemiological and control issues. The taxa *R. puillus, R. rosssicus* and *R. turanicus* s.s. are formally well defined and phylogenetically represent independent lineages, but the biological significance of the phylogenetic relationship among *R. guilhoni, R. camicasi, R. leporis* and the so called “tropical lineage from America” remains unresolved. The taxonomic status of the tick populations that have been called *R. turanicus* in parts of the Mediterranean basin of Spain, Portugal and France and in southern Switzerland also remains unresolved. In fact. they don”t belong to *R. turanicus* s.s. The taxonomic reassessment of species belonging to the *R. sanguineus* group has epidemiological relevance because it has been shown that there are relevant interspecific differences regarding ecological aspects and the vectorial competence to transmit tick-borne pathogens.

O22

Termination of alternative pathway activation by binding of Factor H and FHL-1 facilitates complement resistance of *Borrelia mayonii*

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Introduction: *Borrelia mayonii* has recently been identified as a human pathogenic genospecies causing Lyme disease in the US. Current data reveals a higher spirochaetemia in the blood of patients compared to individuals infected by *B. burgdorferi* suggesting that this genospecies exploit strategies to overcome innate immunity.

Objectives: In this study, we elucidate the molecular mechanisms of immune evasion of *B. mayonii.*
Materials & methods: Serum susceptibility of *B. mayonii* was elucidate by a bactericidal assay and a serum adsorption assay was used to evaluate binding of FH and FHL-1. Furthermore, the factor I-mediated C3b-inactivating activity of FH was analyzed by Western blotting. In addition, biochemical analyses were investigated to characterize the FH-binding protein, CspA_Bmayo. Finally, a gain-of-function strain was generated to verify the role of CspA_Bmayo in facilitating serum resistance of *Borrelia*.
Results: Applying serum bactericidal assays, we showed that *B. mayonii* resists complement-mediated killing. Further experiments revealed that spirochetes acquired FH and FHL-1. In addition, FH retained its factor I-mediated C3b-inactivating activity when bound to spirochetes. Employing bioinformatics, we identified a gene exhibiting 60% identity to the *cspA* encoding gene of *B. burgdorferi*. Our functional analysis revealed that the CspA ortholog CspA_Bmayo interacted with FH and FHL-1. In addition, CspA_Bmayo counteracted complement activation by inhibiting the alternative pathway but not the classical and the Lectin pathway. To elucidate the role of CspA_Bmayo toward complement resistance, a *B. garinii* strain unable to produce any FH-binding proteins was transformed with a vector directing production of CspA_Bmayo. In contrast to the wild-type *B. garinii* isolate, the gain-of-function strain survived in human serum.
Conclusion: This is the first report describing an immune evasion mechanism utilized by *B.mayonii* to resist complement-mediated killing.

O23
Core genome phylogenetic analysis of the avian-associated *Borrelia turdi* indicates a close relationship to *Borrelia garinii*

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Borrelia burgdorferi sensu lato comprises a species complex of tick-transmitted bacteria that includes the agents of human Lyme borreliosis. *Borrelia turdi,* a genospecies of this complex, exists in cryptic transmission cycles mainly between ornithophilic tick vectors and their avian hosts. The species was originally discovered in Asia but has increasingly been found in Europe. Next generation sequencing was used to sequence the genome of *B. turdi* isolates (n=3) obtained from ticks feeding on birds in Portugal to better understand the evolution and phylogenetic relationship of this avian and ornithophilic tick-associated genospecies. Here we use draft genomes of these *B. turdi* isolates for comparative analysis and to determine the taxonomic position within the *B. burgdorferi* s.l. species complex. The main chromosomes showed a maximum similarity of 93% to other *Borrelia* species whilst most plasmids had lower similarities. All three isolates had nine or 10 plasmids and, interestingly, one linear plasmid with a novel partitioning protein; this plasmid was termed lp30. Phylogenetic analysis of multilocus sequence typing housekeeping genes and 113 single copy orthologous genes revealed

that the isolates clustered according to their classification as *B. turdi*. In phylogenies generated from these 113 genes the isolates cluster together with other Eurasian genospecies and form a sister clade to the avian associated *B. garinii* and the rodent associated *B. bavariensis*. These findings show that *Borrelia* species maintained in cryptic ecological cycles need to be included to fully understand the complex ecology and evolutionary history of this bacterial species complex.

O24
Importance of birds in the circulation of neuroinvasive strains of *B. garinii*

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2017 – *B. burgdorferi* s.l. forms a complex of at least 21 genospecies out of which 8 are present in Europe. All the genospecies of *B. burgdorferi* s.l. are maintained via zoonotic transmission cycles involving vertebrate reservoir hosts and ixodid ticks. The importance of avian hosts for the maintenance of borrelial infection is now indisputable. The main aim of the research was to identify the prevalence and genetic structure of *B. burgdorferi* s.l. in association with birds as reservoir hosts. The feeding ticks were collected from the birds during spring and fall migration as well as during nesting season in 2017 and 2018 in Drienovec, Slovakia. Questing ticks were collected by flagging the vegetation each time the birds were checked. In total, 502 local as well as migratory birds that belonged to 40 species were examined for the tick infestation in 2017. 357 *I. ricinus* ticks were collected from 15 species of birds. 7 species of birds carried *Borrelia* infected ticks and 44.4% of collected ticks were infected. Black birds had the highest reservoir capacity and 61.4% of ticks carried *B. burgdorferi* s.l. *B. garinii* and *B. valaisiana* predominated representing more than 94% of all bird associated Borrelial genospecies. On the other hand 67 of 255 (26.3%) questing ticks carried *B. burgdorferi* s.l. that was represented by five genospecies. To better understand the importance of birds on the dispersion of Borrelia, *B. garinii* from bird feeding as well as questing ticks were further analysed by multilocus sequence typing (MLST). MLST analysis of concatenated sequences revealed its high intraspecific variability. Two unique sequence types were described. Moreover, *B. garinii* ST, that were isolated from patients with neuroboreliosis, were detected in bird feeding ticks. Using MLST we have proved the importance of black birds as an important reservoir hosts of neuroinvasive strains of *Borrelia garinii*.
2018 – This study was supported by APVV 16-0463.

O25

Host-parasite interactions between *Borrelia burgdorferi* s.l. and its avian reservoir hosts

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Borrelia burgdorferi sensu lato (s.l.) is maintained in enzootic cycles in nature by vertebrate reservoir hosts, including mammals, lizards and birds. To understand the eco-epidemiology of Lyme borreliosis it is necessary to evaluate the relationships among *Borrelia* genospecies, their tick vectors and vertebrate reservoir hosts. We surveyed infection prevalence in avian hosts and using wild birds as models, we performed transmission experiments, assessed the physiological impact of infection in reservoir hosts and how exposure to stress could affect the host"s infectivity to vector ticks. Additionally, we evaluated the population structure of an avian-associated *Borrelia* genospecies. Thrushes (*Turdus* spp.) were the most important birds in the enzootic cycle of *Borrelia*. The most common genospecies detected in ticks from birds, *B. garinii*, showed no population structure within Europe, which may be related with dispersal and migratory movements of their hosts. The ubiquitous blackbird *Turdus merula* successfully transmitted *B. turdi*, *B. valaisiana* and *B. burgdorferi* s.s. to xenodiagnostic ticks, and the generalist tick *Ixodes ricinus* was found to efficiently vector *B. turdi* and *B. valaisiana*, increasing the risk of exposure of other hosts to genospecies circulating in birds. Oxidative balance (protein carbonyls and glutathione peroxidase levels) of naïve blackbirds was affected by experimental infection with *B. burgdorferi* s.l., suggesting that these bacteria may inflict non-negligible physiological harm on its natural reservoir hosts with potential impact on transmission success. However, there was no evidence that exposure to stressors increased infectivity of wild avian hosts to vector ticks in an experiment performed in captivity.

O26
The genus *Borrelia* reloaded

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The genus *Borrelia*, originally described by Swellengrebel in 1907, contains tick- or louse-transmitted spirochetes belonging to the relapsing fever (RF) group of spirochetes, the Lyme borreliosis (LB) group of spirochetes and spirochetes that form intermittent clades. In 2014 it was proposed that the genus *Borrelia* should be separated into two genera; *Borrelia* Swellengrebel 1907 emend. Adeolu and Gupta 2014 containing RF spirochetes and *Borreliella* Adeolu and Gupta 2014 containing LB group of spirochetes. Here, we describe an analysis based on a method that has been proposed for bacterial genus demarcation, the percentage of conserved proteins (POCP) (Qin et al. 2014), and we included RF group species, LB group species and two species belonging to intermittent clades, *Borrelia turcica* Güner et al. 2004 and *Candidatus* Borrelia tachyglossi Loh et al. 2017. These analyses provide new evidence that all groups of spirochetes belong into one genus and we propose re-unite all groups in the genus *Borrelia*.
Reference: Qin Q-L, Xie B-B, Zhang X-Y, Chen X-L, Zhou B-C, Zhou J, et al. A Proposed Genus Boundary for the Prokaryotes Based on Genomic Insights. Journal of Bacteriology. 2014; 196(12):2210-5.

O27
Phyloproteomic and functional analyses do not support a split in the genus *Borrelia* (phylum Spirochaetes)

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Background: The evolution of species is frequently derived from molecular sequences, and the resulting phylogenetic trees do not include explicit functional information. Here, we assessed the functional relationships among bacteria in the Spirochaetes phylum, based on the biological processes (BP) of 42,489 proteins in reference proteomes. We tested the hypothesis that the species in the genus *Borrelia* were sufficiently different to warrant splitting them into two separate genera.
Methods: Data from 42 reference Spirochaetes proteomes (including free-life species, pathogens transmitted without vector and pathogens transmitted by vector) were annotated using Gene Ontology. Multivariate studies on the molecular processes, classic phylogenetic analysis of presence/absence of proteins and network analysis of the proteins and processes were used to evaluate the hypothesis that the genus *Borrelia* cannot be split in two separate entities.

Results: A detrended canonical analysis showed that the presence/absence of BP contained a strong phylogenetic signal, which did not separate species of *Borrelia*. Analysis of the top ten BP with more proteins revealed that species in *Borrelia* were more similar to each other than to any other Spirochete. A dendrogram based on the presence/absence of proteins demonstrated that distances between species of *Borrelia* were smaller than the distances between species in any other group of Spirochetes. A phyloproteomic network showed a close functional association between species of *Borrelia*. Compared to the other Spirochetes only few proteins were found exclusively in *Borrelia*. All Spirochetes including *Borrelia* share a core proteome. The presence of unique BP or proteins in *Borrelia* was very low, compared to other genera of Spirochaetes.
Conclusions: We found only marginal functional differences among *Borrelia* species. The results did not support a split of the arthropod-transmitted spirochaetes into the proposed genera, *Borrelia* and *Borreliella*.

O28

Molecular mechanisms of inhibition of tick-borne encephalitis virus by nucleoside analogues and neutralization by a monoclonal antibody

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Tick-borne encephalitis (TBE) is a potentially lethal neuroinfection in humans, caused by TBE virus (TBEV), a member of genus *Flavivirus*, family *Flaviviridae*. The disease is prevalent in forested areas of Europe and northeastern Asia. Specific anti-TBEV immunoglobulin is currently used with well-documented effectivity for post-exposure prophylaxis and TBE treatment in Russia and Kazakhstan, but the use of specific TBEV immunoglobulins has been discontinued in Europe due to concerns regarding antibody-dependent enhancement (ADE) of infection in naïve individuals. However, the mechanism of TBEV antibody-mediated neutralization and/or ADE remains unknown. Here, we present cryo-EM structures of the native TBEV virion (strain Hypr, European TBEV subtype) and its complex with Fab fragments of neutralizing antibody 19/1786 at near-atomic resolution. Flavivirus genome delivery depends on membrane fusion that is triggered at low pH. The virion structure indicates that the repulsive interactions of histidine side chains, which become protonated at low pH, may contribute to the disruption of heterotetramers of the TBEV envelope and membrane proteins and induce detachment of the envelope protein ectodomains from the virus membrane. The Fab fragments bind to 120 out of the 180 envelope glycoproteins of the TBEV virion. Unlike most of the previously studied flavivirus-neutralizing antibodies, the Fab fragments do not lock the E-proteins in the native-like arrangement, but but prevented the virus proteins from inducing membrane fusion in the endosome and releasing the viral nucleocapsid into the cytoplasm. Because the IgG 19/1786 antibody is not cross-reactive against other flaviviruses and efficiently neutralizes TBEV, it has potential for therapeutic use in patients with TBE.
Next to immunotherapy, treatment with small molecules interfering with virus life cycle represents another promising approach how to manage TBE in humans. However, there are no small molecule

drugs approved for TBE treatment at present. We tested libraries of diverse molecules for their ability to inhibit TBEV replication. Nucleoside analogues showed the highest anti-TBEV activity both *in vitro* and *in vivo*. Our data demonstrate a relatively stringent structure-activity relationship for modifications at the 2', 3', and 4' nucleoside positions of the nucleoside. Whereas nucleoside derivatives with the methylation at the C2' position or azido modification at the C4'position exert a strong TBEV inhibition activity and low cytotoxicity in vitro, substitutions of the O2' and O3' positions lead to a complete loss of anti-TBEV activity. Treatment of TBEV-infected mice with 7-deaza-2"-C-methyladenosine (7-deaza-2"-CMA) substantially improves disease outcome, increases survival, and reduces signs of neuroinfection and viral titers in the brain. TBEV-resistance to the C2' methylated inhibitors or galidesvir (BCX4430) was found to be conferred by a single conservative mutations that cause a subtle atomic effect within the active site of viral NS5 RdRp, but are associated with strong attenuation of the virus. Nucleoside analogues have, next to immunotherapy, promising therapeutic potential for treatment of TBEV infection.

O29

Pathogenic potential of tick-borne symbiotic *Rickettsia species*

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Many species of *Rickettsia* are considered non-pathogenic solely because they have not been identified in human patients. However, the number of recognized tick-borne rickettsial pathogens is increasing and there is evidence that some of putatively endosymbiotic bacteria can be transmissible from ticks to vertebrate hosts. Transmissibility of these bacteria may complicate recognition, diagnosis and surveillance of rickettsioses in humans; yet there is little information regarding their virulence and pathogenicity. We aimed to assess the ability of 3 putatively endosymbiotic rickettsial species (*Rickettsia amblyommatis*, *R. bellii* and *R. montanensis*) to cause generalized infection, clinical signs, and seroconversion in model animals.
Guinea pigs were exposed to 3 *Rickettsia* sp. by IP-inoculation and infestation with infected ticks. Clinical signs were monitored for 14 days. Generalized infection was assessed by PCR in skin biopsies and internal organs. Seroconversion was measured by IFA using species-specific antigens. For comparison, a group of guinea pigs was infected with *R. rickettsii*.
Clinical signs noted in all animal groups ranged from mild (*R. amblyommatis* and *R.* bellii) to severe (*R. montanensis* and *R. rickettsii*). Exposure to each bacteria resulted in seroconversion with differences in the proportion of seropositive animals and IFA titers. Following IP inoculation, rickettsial DNA was also detected in ear biopsies in all animal groups, but after tick-exposure, only *R. amblyommatis*, *R. montanensis,* and *R. rickettsii* DNA was detected in the skin. The same 3 agents were also detected with varying frequency in the internal organs of exposed animals.
Our results prove that at least 2 species of putatively endosymbiotic tick-borne *Rickettsia* - *R. amblyommatis* and *R. montanensis* cause documentable infections in model animals. Exposure to these agents, rather than to *R. rickettsii*, is likely to be the cause of large numbers of subclinical seroconversions in the US.

O30

Phlebovirus-like sequences detected in ticks and mosquitoes collected in the Russian Federation

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Phlebovirus is abundant and rather heterogeneous genus within *Phenuiviridae* family (order *Bunyavirales*). Members of the genus are associated with a variety of human diseases ranging from mild to severe. It is believed that phleboviruses will continue to be agents of public health importance throughout the current century.
In the last decade large diversity of novel phleboviruses has been reported worldwide. On the other hand phlebovirus endogenous viral elements have been described in various arthropod genomes. The aim of the present study was to investigate a diversity of phleboviruses circulating in the Russian Federation. We used a pan-phlebovirus RT-PCR (Klimentov et al., J Virol Methods. 2016 Jun; 232:29-32.) based on multiple degenerate primers targeting the polymerase gene fragment. Arthoropod specimens were collected in 2005 – 2017. Totally 5648 *Ixodidae* ticks combined into 1017 pools and 2376 mosquitoes combined into 281 pools were screened.
Specific amplicons were produced from pools of *Ixodes persulcatus (*27 pools*)*, *Dermacentor reticulatus* (90)*, Hyalomma scupense* (19), *H. marginatum* (1), *Rhipicephalus rossicus* (1), *Rh. sanguneus* (4), *Rhipicephalus sp* (4), as well as 2 mixed pools of *D. reticulatus* and *D. marginatus*. Positive tick pools originated from republics of Karelia (22 pools), Tatarstan (46) and Tuva (8); Krasnodar (25) and Stavropol Krai (4); Astrakhan (19), Voronezh (2), Kaluga (1), Moscow (2) and Ulyanovsk (22) regions. Four positive pools of *Aedes communis* and one pool of *Aedes dianteus* mosquitoes were detected from Republic of Karelia and St. Petersburg, respectively.
Direct sequencing of amplicones and subsequent phylogenetic analysis revealed nine novel phleboviruses and one new representative of genus *Phasivirus*. Affiliation of detected sequences to either exogenous or endogenous viruses will be discussed.

O31

Zoonotic potential of species in the genera *Anaplasma* and *Ehrlichia*

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Genus *Anaplasma*
Anaplasma phagocytophilum may cause disease in several mammalian species especially in Asia, Europe and North America and is the most important zoonotic infection in the genus *Anaplasma*. Severe symptoms in humans has been reported, especially in the USA and China. Besides *Ixodes* spp., other tick species such as *Dermacentor marginatus*, *Haemophysalis punctata, Hyalomma marginatum, Rhipicephalus bursa* and *R. sanguineus* may occasionally be involved as vectors. Anaplasmosis caused by *A. ovis* has a worldwide distribution in small ruminant populations. One human case have so far been detected (Europe). The infection is spread by *Rhipicephalus* and *Dermacentor* species. *A. carpa* has recently been identified in Asia causing severe human illnesses. Goats are supposed to be the main domestic reservoir. Known vectors are *I. persulcatus* and *Ha. longicornis*. In addition, human disease has been reported in connection with an *A. platys* infection.
Genus *Ehrlichia*
Ehrlichia chaffeensis seems to have a worldwide distribution with a reservoir associated with wild ruminants and is the most important zoonotic infection among *Ehrlichia* species. Severe human infection has been reported in the USA. The infection is mainly associated with *Amblyomma americanum* and *D. variabilis*. *E. ewingii* may cause severe human disease and is reported especially from the USA. The main vectors seems to be *A. americanum*, *D. variabilis* and *R. sanguineus*. *E. muris* / *E. muris*-like agent has been associated with human disease both in the USA, Russia and in eastern Asia. Ticks in genus *Ixodes* are involved. Mild human infection due to PME (Panola Mountain *Ehrlichia*) has recently been recorded (USA), connected to *Amblyomma* spp. In addition, heartwater a disease in ruminants caused by *E. ruminantium,* may cause severe human infection. The infection is associated with different *Amblyomma* species. At last, *E. canis*-like agent has been isolated from human patients.

O32

Investigating small and medium-sized mammals to identify potential reservoirs of *Borrelia miyamotoi* in the North Central U.S.A.

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Introduction: *Borrelia miyamotoi* was first discovered over two decades ago, but the enzootic cycle is still undefined. Several rodent species have been suggested to be reservoir hosts for *B. miyamotoi*. However, their roles or contributions on *B. miyamotoi* maintenance are mostly unknown.

Objectives: We investigated mammal hosts to identify potential reservoir hosts for *B. miyamotoi* maintenance by providing field information required to describe "reservoir potential" and "reservoir competence".
Materials & methods: We trapped mammals during 2010-2012, and screened ear tissue, blood, and attached ticks for *B. miyamotoi* infection by qPCR. Tick loads and the seasonal activity of *I. scapularis* collecdted from captured animals were analized.
Results: From 2152 captures, white-footed mouse was the most frequently trapped host species. Likewise, the majority of larval (72.2%) and nymphal (50.7%) *I. scapularis* were recovered from white-footed mice. The highest mean larval loads were observed from white-footed mice (5.1 ticks/capture), whereas eastern chipmunks (2.6 ticks/capture) harbored the highest mean nymphal loads. *Borrelia miyamotoi* infection was most frequently observed from white-footed mice, with 1/705 tissue and 12/444 blood samples infected and eastern chipmunks showed highest infection prevalence (18.8% blood samples, n=16).
Conclusion: Results suggest low or limited reservoir competence of white-footed mice for *B. miyamotoi* and confirm those from the Northeast that blood appears to be more sensitive compared with ear tissue for detecting *B. miyamotoi*. One species to further investigate is the eastern chipmunks as it showed the highest infection prevalence (18.8% blood samples, n=16) and typically feeds many juvenile ticks. Host infection with *B. miyamotoi* peaked in June, which is several months earlier than that published in the Northeast.

O33

The Tick-Borne-Diseases STING-study – Clinical outcome, epidemiology and prevalence of tick-borne pathogens in Scandinavia

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The distribution of ticks, mainly *Ixodes ricinus*, and tick-borne microorganisms have increased during the last decades, likely due to effects of climate change. To obtain a covering approach to investigate the risk of contracting a disease from a bite of an infected tick in the Scandinavia, we decided to set up a multicenter, prospective follow-up study, the tick-borne diseases (TBD) STING study, in which healthy volunteers are recruited.
The main objectives of the present study is to determine the risk of contracting an infection after a tick bite, and to explore the clinical outcome.
Healthy tick-bitten volunteers are recruited and asked to detach the tick, deliver it to a health care center, deliver a blood sample and to fill in a questionnaire. Three months later, the follow up includes new blood delivery and a new questionnaire. During 2008-2015, we have recruited around 5 500 persons, who delivered over 7 000 ticks, around 10 000 blood samples and the same number of

questionnaires, from over 60 health care centers in Sweden, Finland and Norway. Serum samples were analysed by IFA/ELISA and by molecular methods, respectively for exposure of different tick-borne pathogens: *Borrelia* spp, *Anaplasma phagocytophilum*, spotted-fever *Rickettsia*, *Candidatus* Neoehrlichia mikurensis, *Bartonella henselae*, *Francisella tularensis*, the TBE-virus and the parasite *Babesia spp*. The ticks were analysed for the above mentioned pathogens using molecular methods. From the questionnaires, supplemented with the medical records, we obtained information about the clinical outcome.
The results show that the risk of contracting a tick-transmitted disease is very low. The risk of contracting a *Borrelia* infection after a bite with an infected tick is around 2%, although this is the most prevalent pathogen, with a prevalence higher than 25%. The other bacterial, tick-borne infections are rare and symptoms generally mild. Multiple tick-borne infections are very rare.

O34
Can TBE lead to neurodegeneration – a résumé

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Introduction: TBE may take different clinical courses. Based on a retrospective analysis of medical records of 1072 patients we concluded that sequelae (subjective complaints, neurological and psychiatric sequelae) may affect 20.6% of TBE patients. The pathogenesis of different clinical presentations and sequelae development in TBE has not been fully recognized so far. On the basis of our previous studies we suspected that TBE may lead to neurodegeneration.
Objectives: To assess the neurodegeneration process in TBE.
Materials & methods: Two studies concerning TBE patients were conducted. The studies compared patients with mild and severe course of TBE as well as patients with and without sequelae. In the first study NSE and S-100 concentration in serum and cerebrospinal fluid (CSF) in 43 patients were measured by ELISA. In the second: Tau protein concentration in CSF was determined in 35 patients with TBE. In both studies the control group were patients in comparable age with excluded neuroinfection.
Results: NSE CSF concentration was higher in patients with meningoencephalitis than in patients with meningitis and controls. NSE concentration in serum after 14 days and NSE serum sample 2/serum sample 1 ratio was higher in the sequelae group. ROC curve analysis indicated that NSE concentration in serum after treatment differentiates sequelae group from patients who did not develop sequelae. Tau protein concentration after 14 days of treatment was higher in encephalitis group than in control group. Tau protein concentration was higher in the sequelae group.
ROC curve analysis indicates that CSF Tau protein concentration at admission may predict the complicated course of TBE.
Conclusions: Neurodegeneration process is present in TBE encephalitis, even after clinical recovery. NSE could be used in the prediction of TBE course. Tau protein concentration may be used as a predictor of sequelae development in TBE.

O35
Risk factors and tick-borne encephalitis spatio-temporal variation – a case study in northern Italy

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Introduction: Tick-borne Encephalitis (TBE) is a zoonosis of public health relevance in many European countries. Italy is considered at low risk of incidence, although in some endemic areas (foci the risk of TBE infection is high. The circulation of the TBE virus is characterized by a high variability both in term of location and impact. In the Province of Trento (Italy) the incidence rate had a 5-fold increase in the last six years (2012-2017) compared to the 20 years before (1992-2011), and also the geographical distribution of local foci experienced a northward shift.
Objectives: Our aims were to determine the factors affecting TBE virus circulation among rodent hosts; assess the prevalence of TBE virus in questing ticks in order to estimate the current TBE infection risk in the study area.
Materials & methods: We investigated the pattern of tick infestation and TBE virus seroprevalence in a population of yellow-necked mouse, *Apodemus flavicollis*, in a known TBE focus in the Province of Trento (Italy). We also performed a wide screening of TBEV prevalence in ticks by real-time RT-PCR.
Results: The number of co-feeding ticks on rodents and TBE seroprevalence were affected by the combination of climatic condition, in particular the autumnal cooling, with the abundance of the feeding tick hosts, in particular rodents and deer. The screening of questing ticks for TBE virus confirmed the same very low prevalence which has been previously recorded in the area . Sequencing of the virus from the positive ticks showed that the Western subtype of TBE virus is circulating in this area.
Conclusion: Complex factors shape the pattern of TBE disease focal distribution. Variation of climatic condition, tick host abundance and feeding interactions could affect the circulation and presence of the virus shaping its distribution at local scale. More information on the new TBE foci are needed also in the public health perspective point of view as TBE vaccination could be implemented.

O36
Tick-borne encephalitis in Siberia – statistics, incidence, association of the disease severity and virus genotype

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Tick-borne encephalitis (TBE) is the most severe neuroinfection transmitted by *Ixodes* ticks in Eurasia. Several thousand cases of TBE are recorded every year, with over half the cases, in Russia, where TBE is registered in 48 provinces. The highest incidence of TBE is recorded in Siberian region, which includes 12 provinces. In ten Siberian provinces, the incidence of TBE is significantly higher than

the average incidence in endemic provinces in Russia (1.39). Notably, in five Siberian provinces, the endemic area of TBE has increased in recent years. Despite the increased number of recorded tick bites, the incidence of TBE is constantly decreasing in Russia from the beginning of this century. However, 62% lethal cases are recorded in Siberian region, where total population is small. Three subtypes of TBE virus are detected in this region, but to date, there is no data on which subtype of TBE virus causes the disease more often.
A total of 2006 clinical samples (sera and CSFs) were collected from 1120 adult patients hospitalized with tick-borne infection from the end of April to September, 2015-2018. The samples were screened for the nucleic acids of TBE virus and other tick-transmitted pathogens using RT-PCR or nested PCR (bacterial agents). In addition, TBE was diagnosed using ELISA of patient"s sera. Approximately one third of patients with confirmed tick-transmitted infection had TBE. All samples positive for the nucleic acid of TBE virus were sequenced and viral subtype was determined. Association of the subtype of TBE virus and clinical manifestations, severity and outcome of the disease has been analyzed. We hypothesize that high diversity of TBE virus genetic variants circulated in Siberia could explain in part the higher than average incidence rate of TBE and variable clinical symptoms/syndromes of the disease in this region.

O37

Host genetic control of tick-borne encephalitis virus infection – current progress and future prospects

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Introduction: The result of a host-virus interaction (and the course and outcome of a viral disease) largely depends on individual peculiarities of the host (particularly, human) genome, that determine the ability of the immune system to suppress the development of a viral infection. Unlike other infections caused by viruses from the Flaviviridae family (Dengue fever, West Nile fever, hepatitis C), the host genetic control of tick-borne encephalitis (TBE) virus infection has been rather poorly studied to date.
Objectives: Search for human genetic factors associated with predisposition to TBE in a Russian population.
Materials & methods: DNA samples from 177 non-immunized Russian TBE patients, including patients with severe paralytic forms (meningoencephalitis, etc.) ($n = 65$) and with milder fever ($n = 51$) and meningitis ($n = 61$), as well as 215 DNA sample from the control population, were used in this study. Both candidate gene approach and genome-wide association studies (whole exome sequencing) were applied to search for single nucleotide polymorphisms (SNPs) in human genes associated with predisposition to TBE.
Results: To date, we identified 13 SNPs located within nine human genes (including the oligoadenylate synthetase 2 (*OAS2*) and 3 (*OAS3*), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (*CD209*), toll-like receptor 3 (*TLR3*), interleukin 28B (*IL28B*), interleukin 10 (*IL10*), matrix metalloproteinase 9 (*MMP9*), ATP-binding cassette sub-family B member 9 transmembrane transporter (*ABCB9*), and XXII type collagen (*COL22A1*) genes) associated with predisposition to

TBE in the Russian population. Most of the above genes encode proteins that are crucial components of the innate immune system.
Conclusion: Identification of the genes involved in the development of human predisposition to TBE allows to clarify the mechanisms of host genetic control of TBE virus infection.
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O38
Tracking the route of *Borrelia afzelii* transmission from infected *Ixodes ricinus* nymphs

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Quantitative tracking of *Borrelia afzelii* has shown that its transmission cycle differs from the *B. burgdorferi* transmission by *Ixodes scapularis*. *Borrelia afzelii* are abundant in the guts of unfed *Ixodes ricinus* nymphs and their numbers continuously decrease during feeding. In contrast, spirochetes are not present in the salivary glands. *Borrelia afzelii* transmission starts during the early stages of feeding, spirochetes could be detected in murine skin within 1 day of tick attachment. Tick saliva is not essential for *B. afzelii* infectivity, the main requirement for successful host colonization being a change in outer surface protein expression that occurs in the tick gut during feeding. Spirochetes in vertebrate mode are able to survive within the host even if the tick is not present. On the basis of our data we hypothesize that a direct migration of spirochetes from midgut to the mouthpart appears to be a possible route of *B. afzelii* transmission.

O39
Phenology of *Ixodes ricinus* and its infection rates with *Borrelia burgdorferi* in the Netherlands – results of ten years of monthly collections

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Question: Ten years of monthly sampling of ticks by volunteers has resulted in a longitudinal dataset that allows us to study a number of research questions about the spatial and temporal variation in the density of questing *Ixodes ricinus*, the borrelia infection rate and the habitat and climatic factors that may be determining this variation.

Methods: Ticks of all mobile life stages were collected on fixed transects in eleven locations throughout Netherlands (2006-2016). Nymphs were tested for *Borrelia burgdorferi*. We used GLMMs to investigate the effect of climatic and vegetation-related variables on the abundance and timing of activity.
Results: Tick numbers increased slightly over ten years, but trends differed between locations. We did not find significant effects of temperature or humidity on the total annual numbers of ticks, but we did find significant differences between locations. Relevant habitat factors could be humus layer thickness, the abundance of moss, and food plants for rodents (blackberry and blueberry). The onset of questing activity differed markedly between locations, and was correlated with temperatures in winter and early spring. Another important finding is that ticks are sometimes active in winter in The Netherlands. We show the spatial (between locations) and temporal (between year) variation in infection prevalence, as well as a clear seasonal trend.
Conclusion: We conclude that climatic variables do not have a straightforward association with tick density in the Netherlands, but that winter and spring temperatures influence the onset of tick activity. Several habitat factors, such as abundance of blueberries and thickness of litter layer seem to be associated with tick densities. We found typical infection rates of 10-30 % in ticks, with some variation between locations and between years. This type of longitudinal study is essential in improving our understanding of the ecology of Lyme borreliosis.

O41

A pan-European overview on the surveillance, reporting, data availability and laboratory capacity for Lyme Borreliosis

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In Europe, Lyme borreliosis is endemic but the quantification of infection prevalence and incidence remains challenging. The causes are complex, but most of the unreported cases are related in principle to the lack of diagnostic, over-complicated reporting procedures or lack of mandatory reporting. Existing official data and literature information is plagued by the heterogeneity of clinical outcomes and the use of different laboratory criteria for diagnosis. We aimed to review the situation on the surveillance, reporting, epidemiologic data availability, and laboratory capacity and diagnostic methods for Lyme borreliosis as well as the NUTS3 presence/absence of reported human Lyme borreliosis cases in the European Union, European Economic Area and Balkan. The information was collected using a dedicated questionnaire, in the frame of a multinational project coordinated by ECDC. Out of 38 countries, 30 have responded. Seven countries reported changes in the reporting, surveillance or type of data available in the national records for the period 2011-2015. The reporting of Lyme borreliosis was mandatory for the whole period 2011-2015 in 18 countries. Since 2012, the reporting is mandatory also in Ireland. The reporting referred exclusively to Lyme borreliosis in 12 countries. 28 countries reported no changes on the surveillance

system for the period 2011-2015. Out of these, three reported “no surveillance”, 17 reported “comprehensive surveillance” and 10 reported “other types of surveillance”. Among the ten countries, most of the countries which reported “other type of surveillance” use a sentinel system. The epidemiological data is collected “case-based” in 18 countries, “aggregated” in three countries while ten countries are not collecting data. 16 countries have and 14 don”t have reference laboratories for Lyme borreliosis. Additionally, out of the enquired 38 countries, 34 provided responses on the presence/absence of human cases of LB for each year (2011-2015).

O42

Genetic variability of *Dermacentor reticulatus* in Europe

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Introduction:. *Dermacentor reticulatus* ticks are an important tick-borne disease vector in Europe transmitting *Rickettsia* spp., TBEV, *Borrelia burgdorferi* s.l., *Anaplasma phagocytophlium, Bartonella* spp. *Coxiella burnetti*, *Francisella tularensis* pathogens. It is observed that habitable range is expanding into Northern Europe, reports also include regions of Southern Europe where distribution is overlapping with *Dermacentor marginatus*. The expansion pattern is not yet definitively described as there are unanswered questions regarding distribution gap in Central Europe. This gap could be already inhabited and the migration of individuals quite frequent.
Objectives: The aim of this study was to investigate *D. reticulatus* genetic diversity and population structure over a large geographical area in different zones of distribution.
Materials & methods: In this study we used five microsatellite marker assays, sequencing of 12S, 16S rRNA gene fragments and ITS2 to investigate *D. reticulatus* genetic variability across Europe. Samples were collected from most of the tick distribution range including Spain, Great Britain, France, Germany, Poland, Baltic States, Slovakia and extending to Ukraine and Kazakhstan.
Results: Polymorphism of microsatellite markers were analyzed by calculating number of alleles, observed and expected heterozygosity inbreeding coefficient and determining of genetic grouping by Bayesian-clustering method. Gene fragment sequencing analysis were performed by investigating number and diversity of haplotypes, nucleotide variability, number of polymorphic sites, constructing of phylogenetic trees and haplotype networks. From this study we were able to investigate genetic variability and spreading patterns of *Dermacentor reticulatus* ticks.
Conclusion: Division of *D. reticulatus* distribution range into Eastern European and Western European populations with a possible recent overlap in Poland has been supported by molecular data obtained in this study.

O43
Population genetics of *Dermacentor variabilis* in the United States

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Introduction: *Dermacentor variabilis* is a North American ixodid tick widely distributed in the United States eastwards of a line drawn from Montana to South Texas, and a few, to some extent, isolated populations along the west coast. *D. variabilis* commonly bites humans, and is the main vector of *Rickettsia rickettsii*, the etiological agent of Rocky Mountain Spotted Fever in the central-eastern US. Studies directed to the population genetic structure of this tick species are scarce, and are mostly based on a single mitochondrial gene marker. These studies demonstrated two phylogenetic clades, one corresponding to the west coast populations (western clade), and the other including all populations eastern of the Rockies (eastern clade). It has been hypothesized that both groups may correspond to different species.
Objective: The aim of this study was to investigate the population genetic structure and potential speciation of *D. variabilis*.
Materials & methods: To do this we generated a new data set based on nuclear markers, single nucleotide polymorphisms (SNPs), discovered through next generation sequencing.
Results: The results showed moderate population structure, and supported the occurrence of gene flow between some genetic clusters. Maximum parsimony phylogenetic reconstructions showed a divergent and monophyletic western clade, and a generally eastern clade.
Conclusions: Overall, the nuclear data set analyzed herein is congruent with previous findings based on mitochondrial markers, although it led to a higher level of resolution within the eastern clade. Additional lines of evidence are needed to determine whether eastern and western populations correspond to different species.

O44
Identification of closely related *Ixodes* species by protein profiling with MALDI-TOF Mass Spectrometry.

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Question: For epidemiological studies of tick-borne diseases, the precise identification of ticks is crucial to define their potential role as vectors, to develop control and prevention strategies. Morphological-based and molecular methods are widely used to identify ticks, more recently MALDI-TOF technology shows a great ability to identify ticks.

The published databases include tick specimens of different genera but in the present study, MALDI-TOF MS technology was assessed for the identification of nine closely related tick species belonging to the *Ixodes* genus.
Methods: Tick specimens were morphologically identified using taxonomic keys and then dissected separating 4 legs and cutting the idiosome. Two to five specimens per species were genotyped using the 16 and the COI genes. All specimens were submitted to the MALDI-TOF MS procedure and the half-idiosoma and the 4 legs were individually tested. The intraspecies reproducibility and the interspecies specificity of the MS profile were evaluated.
Then a database was created using the molecular identified specimens. This database was blindly tested using the other remaining specimens.
Results: A total of 246 ticks specimens were submitted to MALDI-TOF MS. For each compartment, the intraspecies reproducibility and interspecies specificity of the profiles were evaluated and revealed that the prime determinant of the grouping of the spectra was the tick species. Subsequent blind test with both legs and half-idiosoma of the created database by the remaining specimens shown 98.5% of correct identification.
Conclusions: The MALDI-TOF MS by its accuracy, high throughput capability and reagent low cost constitutes a remarkable tool for tick identification and to set up databases for epidemiological studies on tick-borne diseases.

O46

Development of a biological tick trap based on an attract-and-kill strategy – efficacy to attract and catch ticks

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In Europe, *Ixodes ricinus* (Acari: Ixodidae) is the most abundant tick species and the main vector of tick-borne diseases (TBD). Increased public awareness concerning TBD has raised interest in an effective tick control. The aim of this project is to develop a control method against *I. ricinus* based on an attract-and-kill strategy. Therefore, we screened long- and short-range attractants as well as substances causing arrestment in ticks. In behavioural assays using a novel y-olfactometer we performed a broad screening of compounds of the classes of aldehydes, lactones, terpenoids, and others for their attractivity towards *I.ricinus* nymphs. Here we demonstrate a significantly attractive effect of CO2 and acetone on the tick.
A first field test demonstrates the potential to attract and trap *Ixodes ricinus* ticks. Further tests are necessary to evaluate the efficacy of the trap under different ecological and meteorological situations and to determine the range ticks are recruited and trapped.

O47

Comparative efficacy of natural and synthetic acaricides against larvae of *Rhipicephalus sanguineus* Canestrini (Acari: Ixodidae)

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The brown dog tick *Rhipicephalus sanguineus* is the vector of *Babesia canis*, *Ehrlichia canis*, *Hepatozoon canis* and *Rickettsia rickettsii* in Brazil, biopathogens responsible for babesiosis, ehrlichiosis, hepatozoonosis and Brazilian spoted fever, respectively. It is the tick species most commonly found in urban dogs around the world. Studies have demonstrated that ticks have been controlled by chemical acaricides, anti-tick vaccines and other methods being the first one the most used despite tick resistance development to the products and other disadvantages. Herein, we evaluated through the *in vitro* bioassay larval packet test (LPT) the comparative efficacy of Cypermethrin (100, 200, 400 e 800 μg active ingredient - a.i.), Neem oil (2250, 4500, 9000 and 18000 μg a.i.) and Orange oil (1500, 3000, 6000 and 12000 μg a.i.) against the tick *R. sanguineus*. The larvae were from a tick colony maintained at the Federal University of Rio de Janeiro - RJ, Brazil and aged two weeks after hatching from the egg masses. The chemical acaricides were formulated in emulsified concentration for posterior water dilution. Results expressed in mean percentage of larval mortality were as follows: Cypermethrin 100.0 μg a.i. (99,44%), 200.0 μg a.i. (100%), 400.0 μg a.i. (100%) and 800.0 μg a.i. (100%); Neem oil 2250.0 μg a.i. (94,82%), 4500.0 μg a.i. (97,90%), 9000.0 μg a.i. (100%) and 18000.0 μg a.i. (100%), Orange oil 1500.0 μg a.i. (100%), 3000.0 μg a.i. (100%), 6000.0 μg a.i. (100%) and 12000.0 μg a.i. (100%), having the botanical oil induced larval mortality significantly higher than the synthetic acaricides ($p<0.05$). It can be concluded that the botanical oils, more ecofriendly products, were effective *in vitro* against larvae of *R. sanguineus*, however, they should be tested at higher concentrations to achieve an efficacy above 90%.

O48

***Hemolivia mauritanica* infection in *Hyalomma aegyptium* from Corum Province, Turkey**

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The main hosts of *Hyalomma aegyptium* adults are Palearctic tortoises of the genus *Testudo*, and accordingly the distribution of this tick species is restricted to the distribution of its principal hosts. Larvae and nymphs of this species are less host-specific, while nymphs have an affinity to humans. *Testudo*

graeca tortoises were captured by hand in opportunistic encounters by walking through the convenient tortoise habitats in random fields and around human habitations between March and December of 2017 in Corum Province of Turkey. A total of 261 *H. aegyptium* ticks (133 males, 29 females and 99 nymphs) were removed from 26 tortoises. Overall, 51.9% of the ticks were infected with *Hemolivia mauritanica* by PCR. *Hemolivia mauritanica* is the most widely distributed blood parasite of turtles worldwide. The high percentage of this parasite found in *H. aegyptium* ticks confirms the co-evolution theory within the Testudo-Hyalomma-Hemolivia host–parasite complex in previous studies. Many pathogens of medical and veterinary importance have been detected in *H. aegyptium* worldwide. In the present study tortoises were collected around human habitations and accordingly, the public health importance of tortoises, their ticks and pathogens should be farther examined.

O50
Eco-Epidemiology of Crimean-Congo Hemorrhagic Fever (CCHF)

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CCHF is the most widespread tick-borne viral disease of humans. It has been recorded from more than 30 countries [1-3]. Humans acquire the infection via tick bites, crushing of infected ticks or contact with viraemic animals blood or tissues. Nosocomial infections due to contact with infected patients are also common. Still the most important route seems to be tick bites [1, 3].
Ticks are accepted as the reservoirs of the infection. Animals can be viraemic for up to 2 weeks and ticks can harbour the virus lifelong (1 year) and can also maintain it by transstadial transovarial transmission [1, 4]
Although CCHFv has been isolated from about 30 tick species, the vector competence has been demonstrated only for limited number of tick species (*Amblyomma variegatum, Hyalomma marginatum, H. rufipes, H. anatolicum, H. asiaticum, H. truncatum, H. impeltatum, Dermacentor marginatus, Rhipicephalus evertsi, Rh. rossicus*), among which *Hyalomma* species are strictly associated with the global distribution of the disease [1, 4-8]. The tick species associated with the current CCHF epidemic in Turkey is *H. marginatum* [9, 10]. The same species is known to be involved in CCHF outbreaks in Balkans, Crimea and Southern Federal Districts of the Russian Federation. It is also important to mention that *Dermacentor marginatus* may play role in enzootic cycle, in the places where *H. marginatum* is involved in human cases. *Hyalomma anatolicum* in Iran, Pakistan, Turkmenistan and Tadjikistan; *Hyalomma asiaticum* in Central Asia and China, *Hyalomma rufipes* in Africa are suggested as the main vectors of CCHF. It is important to mention that in CCHF cases out of H. marginatum's areal are highly correlated with animal butchering together with tick bites [1, 4, 11].
Hyalomma ticks can transmit the virus transstadially and transovarially [1, 4-7, 12-14] Non-viraemic transmission among co-feeding ticks [7, 15, 16] and venereal transmission have also been demonstrated [15].
Hyalomma marginatum is associated with wildlife and is adapted to steppe climate. It is a 2 host tick which' immatures (larvae and nymphs) feed on small mammals (hare, hedghog) and ground frequenting birds (rooks, patriges).

Fed larvae moult on the host and became nymphs which drop-off the ground when engorge. The feeding period of immatures (larvae, nymphs) takes about 14-26 days. Engorged nymphs moult to adults in about 4-20 days on the ground. The time when newly formed adults appear is about late August-September. Those adults preffer to hide and overwinter in nature and activate in the spring of the following year. The adults of *H. marginatum* are of hunter character and instead of vertical climbing on vegetation they actively seek/wait hosts horisontally on the ground. They mostly prefer artiodactyls (cattle, sheep/goats, horses, wild boars) but can aggressively attack humans as well. When attached to a host they feed for about 9-14 days. Engorged females drop-off on the ground and lay about 7000 eggs before they die. The whole life cycle of *H. marginatum* takes about a year [11, 17-20]. The ability of *H. marginatum* to overwinter is one of the main factors which is allowing of the transmission of CCHF virus from year to year. [1, 18].

Adults of *H. marginatum* are responsible of CCHF infections in humans in the Balkans, Crimea, Sothern Federal Districts of Russian Federation and Turkey. Adult ticks become active in spring when average temperatures reach 10.5°C. They actively seek/wait for a host when average temperatures are 22-27°C and humidity is 75-100%. When air temperature increases above 30°C and soil temperature above 45°C ticks preffer to hide even burry in the soil [11, 20].

CCHF epidemics in Balkans, Crimea, Southern Federal Districts of Russia have been always associated with ecological changes leading to increase of wild animals and *H. marginatum* population [1, 18]. In Turkey first cases were diagnosed in 2002 and until the end of 2018 12000 cases were reported from about 3500 rural settlements. The overall mortality was 4.5%. In Turkey it has been shown that *H. marginatum* is the dominant species in the CCHF areas. [9, 10]. It has been also demonstrated that *H. marginatum* is transmitting the virus both transovarrialy and transstadially. [10, 21] and 16.43% of host seeking *H. marginatum* ticks were CCHFv infected [22].

Tick population dynamics are influenced by biotic and abiotic factors [23, 24]. The habitat suitability maps for *Hyalomma marginatum* were prepared and analyzed against spatial distribution of CCHF in Turkey. The disease risk was strongly associated with presence of *H. marginatum* and landscape fragmentation. [10, 25]. The empirical observations on habitat regeneration and its influences on wildlife and tick abundance still need to be discussed. Hare (for immature ticks) and cattle (for adult ticks) seem to be main hosts involved in the *H. marginatum* biology in the epidemic area in Turkey. Both animals support tick population and are also involved in viral circulation [21]. We still need data on other wild animals such as wild boars, which' population is dramatically increased in the region, and other tick species as well (eg. *D. marginatus)* to explain the enzootic cycle of CCHF in Turkey. Also changes in socio-economics (migration from rural areas to urban areas) and animal husbandry (eg. decrease of sheep population) should be studied in details.

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O51

Ticks and tick-borne pathogens from migratory birds in southern and central Europe

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Introduction: Migratory birds are known carriers for foreign tick species and their associated pathogens. They transport e.g. larvae and nymphs of the genus *Hyalomma* spp., which are dropped along their way. In case of suitable weather conditions at the new spots, those stages are able to molt to the next stage e.g. adults and seek for hosts. So happened at the beginning of October in 2018 in the north of Austria, where an adult *Hyalomma marginatum* was found attached on a Haflinger horse.

Objectives: Limited information is available on the composition of bird species within the migratory birds, the ticks from those birds and the pathogens, which are traveling in the ticks on the birds. Therefore we obtained data in 2016 and 2017 at a bird ringing station on an island in Italy, which is a known resting place for migratory birds on their route.

Materials & methods: We investigated ticks from birds, which were captured in course of the ringing procedure on Ponza Island (Italy). After species determination, the ticks were analyzed for different pathogens such as Crimean-Congo Hemorrhagic Fever virus (CCHFv) and *Rickettsia* spp. by using molecular tools.

Results: From 728 captured birds, 104 birds showed tick infestation and 231 ticks were derived from these. The vast majority of ticks were *Hyalomma* spp. Out of these 20.1 % were tested positive for "spotted-fever-group" Rickettsia comprising *Rickettsia aeschlimannii*, *Rickettsia africae* and *Rickettsia raoultii.* The adult tick from the Haflinger horse also was infected with *R. aeschlimannii*. None of the ticks were found positive for CCHFv.

Conclusion: Migratory birds represent a constant possibility for exotic tick species to be introduced to northern areas. With these tick species new zoonotic pathogens such as *R. aeschlimannii* come along and there is a potential for CCHFv to be introduced as well. The public should be trained to notice "new and exotic" tick species.

O52

Hyalomma marginatum and *Hyalomma rufipes*: What do we know about the biology and ecology of these regular visitors in central Europe?

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Hyalomma marginatum and *Hyalomma rufipes* are two-host ixodid ticks that occur in semi-arid and arid regions mainly in the Mediterranean area as well as around the Black Sea and in different parts of Sub-Saharan Africa, respectively. They are known vectors of Crimean-Congo haemorrhagic fever virus and several other pathogens. The immature stages parasitize small mammals such as hares and rabbits, but also ground-frequenting birds. The main hosts of adults are large mammals, and they may occasionally also parasitize humans.
Altogether, larva and nymph stay on their host for up to 3-4 weeks. As a consequence, numerous *H. marginatum* and *H. rufipes* nymphs arrive attached to migratory birds in central and northern Europe in spring where they sooner or later detach.
In general, temperatures in central Europe are considered to be too low for detached engorged *Hyalomma* nymphs to complete the remaining part of their life cycle as findings of adult *Hyalomma* ticks in central Europe have been rare. This was, however, different in the extremely warm and dry growing season of 2018, when dozens of adult *Hyalomma* ticks were found on large mammals in different parts of Germany. Knowledge on the biology and ecology of these prominent vector tick species is limited, so it is currently not possible to make reliable forecasts about whether or not these species have been able to (i) produce offspring in Germany and (ii) survive into 2019.
This talk will give a brief overview about the known relevant biological features of both *Hyalomma* species and will discuss the chances of these species to establish populations in certain parts of central Europe under changing climatic conditions using a habitat model.

O53

Molecular species discrimination of *Hyalomma* and other ticks potentially transmitting Crimean-Congo hemorrhagic fever virus in sub-Saharan Africa

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Introduction: Crimean-Congo hemorrhagic fever virus (CCHFV) is a wide spread arthropod-borne virus which is distributed in many parts of Africa, Asia and Europe. It is mainly transmitted by ticks of the genus *Hyalomma*. CCHFV can cause severe hemorrhagic fever in humans with case fatality rates of 5 – 30% or higher. The species identification of tick vectors, especially *Hyalomma*, is a prerequisite to understand the epidemiology of viruses like CCHFV and to reveal their vector competence.

Objectives: The main goal of this study was to establish molecular species identification methods, which allow a morphology-independent differentiation of the most common *Hyalomma* species in sub-Saharan Africa (*H. truncatum*, *H. rufipes* and *H. dromedarii*).
Material & methods: All analyzed ticks were collected in Cameroon, Mauritania or came from laboratory colonies. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) based on tick leg proteins and the genetic typing by restriction enzyme digestion were assessed as valid molecular species identification methods. Therefore a PCR targeting the CO1 gene of *Hyalomma* ticks was developed to obtain reference sequences for the evaluation of MALDI-TOF MS and Restriction Fragment Length Polymorphism (RFLP) approaches.
Results: Three different *Hyalomma* species are most prevalent in the study areas from sub-Saharan Africa. Using the restriction enzyme cleavage approach we could unambiguously discriminate them on species level.
MALDI-TOF reference spectra were generated for twelve different hard tick species, among them five *Hyalomma* and seven species of four other genera. Each tick species showed characteristic spectral features and spectra clustered accordingly.
Conclusion: The three *Hyalomma* tick species analyzed in this study can be reliably identified by a combination of morphological and molecular (RFLP and MALDI-TOF MS) methods.

O54
TBE in Germany – Design and first results of the project OSWALD

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Project OSWALD is a Germany-wide research project with the aim to create new risk maps for tick-borne encephalitis (TBE). The main goal is to develop state-of-the-art models that use existing knowledge about TBE and uncover knowledge gaps. The project was divided into 5 working groups. Main emphasis is on the Working Group on Modelling (leading partner: University of Veterinary Medicine Vienna), which develops different spatial and temporal models and defines the requirements for the input data and the necessary knowledge about the underlying processes. The Working Group on Tick Density (leading partner: University of Hohenheim, supported by the Universities of Leipzig, Hannover, Vienna, and the company tick-radar GmbH) coordinates a German-wide flagging initiative to provide standardized monthly tick densities from about 100 locations distributed all over Germany over two years. The Working Group on TBE Virus Prevalence (leading partner: University of Hohenheim) provides TBE virus prevalences in ticks flagged from the vegetation.

The Working Group on Tick Activity (staffed with experts from the companies Insect Services GmbH and tick-radar GmbH) conducts field studies on the questing as well as field and laboratory experiments on the development and diapauses of *Ixodes ricinus*. The Working Group on Host Densities (Universities of Leipzig, Hohenheim, and Vienna) has the task to compile the necessary database for the densities of red deer, wild boar, and hares. The first obtained results of project OSWALD comprise (i) monthly flagging data from the year 2018, (ii) preliminary estimations of the mortality rate of adult *I. ricinus* and the behavioral diapause function of *I. ricinus*, (iii) a Poisson regression model to predict next season's *I. ricinus* density, (iv) a new habitat model to estimate suitable regions for TBE virus circulation, and (v) a SIR-type process model for human TBE cases.

O55

Predicting the TBE and Lyme borreliosis vector *Ixodes ricinus* in space and time

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The castor bean tick *Ixodes ricinus* is the principal vector of viral, bacterial, and protozoan pathogens causing growing public-health issues over the past decades. Most prominent are the tick-borne encephalitis virus and the bacteria complex *Borrelia burgdorferi* s.l. Proper risk assessments for such tick-borne diseases need quantitative spatial and temporal predictions of questing tick densities. Here, we present the first high-resolution density map of questing *I. ricinus* nymphs compiled for Germany. The input dataset comprises mean annually accumulated nymphal density, as observed by monthly flagging of 100 m^2 at 69 sampling sites in Germany. A quasi-Poisson regression model was developed to interpolate the observed tick densities at unsampled locations by using bioclimatic variables and land cover. The root mean square error of RMSE=126 nymphs per 100 m^2 is of the order of the inter-annual variation of the tick densities. While tick densities are very low in urban areas, maximum values up to 1,000 nymphs per 100 m^2 are observed in broad-leaved forests. Exemplary one sampling site was selected to answer the question whether it is possible to predict next year's density by time-lagged abiotic and biotic variables. Cross-correlation maps and a quasi-Poisson regression model were applied for an eight-year time series of nymphal *I. ricinus* flagged in Haselmühl (Germany). The annual density can be forecasted with the mean annual temperature of the previous year, the mean winter temperature (Dec. to Feb.), and the fructification index of the European beech two years prior. This resulted in an explained variance of 93 % and a RMSE of 22 ticks per year. Until now, predictions were published and verified for the last two years. Both the year with a low density of 187 nymphs per 100 m^2 in 2017 and the extraordinary tick year 2018 with a density of 443 nymphs per 100 m^2 were correctly predicted by end of February each year. The tick prediction for 2019 will be presented.

O56

Tick-bite associated dermatitis in an Afro-descendant population living in a tropical region in South West Colombia

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Introduction: According to local healthcare providers tick-borne associated dermatitis seemed to be very common in an Afro-descendant population living in a remote area in South-West Colombia.
Objective: To determine the prevalence and etiology of tick-bite associated dermatitis in three communities.
Method: Two cross-sectional studies were conducted in three villages, the first cross-section at the end of the rainy season and the second 8 weeks after the baseline examination. Blood samples were collected to determine antibodies against *R. rickettsii* and *R.typhii* seropositivity in humans. Ticks were collected and species identified.
Results: The study showed that clinically the observed tick-bite dermatitis resembled a prurigo-like disease, but histopathology showed that it was a classic arthropod immune reaction. Overall, acute lesions were presented in 62.9% (95% CI 56-70) of the population. Prevalence of chronic lesions was 94.6% (95%CI 92-97%). Predilection sides were the lower and upper extremities followed by the abdomen and back. 38% of the patients stated to suffered from severe itching and 29.9% from an itching-associated sleeping disturbance. Antibodies against Rickettsia from the spotted fever group (*R. rickettsi*) were detected in 79.0% (95%CI 73-86) of all cases. No clinical case of rickettsiosis was observed. All ticks were from the species *Amblyomma cajennense*.
Conclusion: Prevalence of tick-bite associated dermatitis was extremely high in this setting. As tick-bites were associated with severe itching and sleep disturbance, life quality of affected individuals was impaired.

057

Why ticks and tick-borne pathogens produce their own α-Gal?

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Question: The objectives of this study were (i) to test whether ticks and tick-borne pathogens were able to synthetize α-Gal, (ii) to test whether tick bites induce anti-α-Gal Abs in dogs and the potential protective role of these Abs against *Anaplasma phagocytophilum* in dogs.

Materials & methods: The *Ixodes scapularis* genome was searched for galactosyltransferases (galt). Heterologous gene expression in α-Gal-negative cells and gene knockdown by RNAi in ticks were used to test the α-Gal-synthetizing activity of selected genes. The presence of α-Gal in *A. phagocytophilum* was measured by flow cytometry and immunofluorescence. Dogs were experimentally infested with *Ixodes ricinus* and levels of anti-α-Gal Abs were measured before and after tick infestation. An epidemiological survey was then performed and correlation analysis was used to test for association between anti-α-Gal Ab levels and *A. phagocytophilum* infection.

Results: Three putative galactosyltransferase genes were identified. α-Gal-negative cells transformed with any of the genes became α-Gal-positive. Gene knockdown in ticks reduced the levels of α-Gal *in vivo*. Triple knockdown showed that these genes are essential for tick feeding. α-Gal was also detected in the surface of *A. phagocytophilum*. Tick bites induce an increase in anti-α-Gal Ab levels in dogs. Significantly higher levels of anti-α-Gal IgM were recorded in dogs negative to *A. phagocytophilum* infection.

Conclusion: Results showed that tick galt are involved in α-Gal synthesis in ticks. *A. phagocytophilum* increases the level of tick α-Gal, which potentially increases the risk of developing AGS after a bite from a pathogen-infected tick. Results suggested a protective effect of anti-α-Gal IgM against anaplasmosis in dogs.

O58

Unlocking the mechanisms of tick salivary gland control, promoting the development of tick and tick-borne disease control measures

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Tick salivary glands play a crucial role in maintaining homeostasis during both on- and off- host periods, while products of their secretions compromise the host"s defence system during the tick"s feeding. Secreting a variety of substances via the complex configuration of distinct salivary gland cell types assumes the necessity of precise and dynamic control mechanisms for this highly versatile organ. Up to date neuronal commands, along with their upstream actions on the autocrine/paracrine signals in this tissue, have been suggested as the primary factors regulating tick salivary secretions. A combination of morphological and physiological approaches confirmed that tick salivary glands are presumably controlled by the orchestration of neuropeptides, catecholamines, and cholinomimetics molecules. Furthermore, various specific axonal projections arising from distinct types of neuronal cells have been shown to be in direct contact with three different types of salivary gland acini. Based on our in-depth observations, it appears that ticks utilize various axonal projections to control different parts/cell types of their salivary gland for direct responses to the challenges faced in the fluctuating environment. Consequently, disrupting the salivary glands activities appears to be the most straightforward approach for the development of new strategies to manage ticks and subsequently tick-borne pathogen transmission. While research continues to shed additional light on the mechanisms of tick salivary gland physiology, successful application of this knowledge in the future is necessary to control this growing risk to human and animal health.

O59

Tick mischief: What can be detected in juvenile ticks *Dermacentor reticulatus* collected from rodents?

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Question. Juvenile *D. reticulatus* ticks cannot be collected from vegetation. To detect vertical transmission of *B. canis*, larvae and nymphs were collected from rodents. Detection of pathogen DNA is sensitive enough to detect not only pathogens vectored by ticks but also these taken with blood meal. We evaluated the extent of such contamination.
Methods. Ticks were collected from 140 rodents: 91 bank voles and 49 *Apodemus* and *Microtus* spp. 504 *D. reticulatus* (266 nymphs, 238 larvae in 50 pools) were studied for presence of *Babesia, Bartonella* and *Rickettsia* DNA. We analyzed effect of host factors on prevalence and compared rate of pos. samples between pathogen-pos. and pathogen-neg. rodents. Blood samples from 49 rodents were studied for the presence of *Babesia* and *Bartonella*.

Results: High rate of PCR-positive samples was obtained for *Rickettsia* spp. (28%) and *R. raoultii* was identified in 22 sequenced products. *Babesia* DNA was detected in 20 *D. reticulatus* nymphs: 18 *B. microti* (6.8%) and 2 *B. canis* (0.8%). *B. microti* DNA was also detected in 4 pools of larvae (8%). Detectability of *B. microti* was higher for typical *B.microti*-hosts as *Microtus* than for *Apodemus* spp. *B. microti* was detected in 68% of nymphs collected from *B.microti*-pos. rodents in comparison to 1.6% of nymphs collected from *B.microti*-neg. rodents.
Bartonella DNA was detected in 18% of *D. reticulatus* (38% larvae pools, 14% of nymphs). Highest rate of positive ticks was collected form *Apodemus* spp., known *Bartonella* reservoir.
DNA of *Bartonella* was detected in 42% of nymphs and 57% of larvae pools collected from *Bartonella*-pos. rodents in comparison to 28% of nymphs and 11% of larvae collected from *Bartonella*-neg. rodents.
Conclusions: Vertical transmission of *B. canis* was confirmed in the field. Meal contamination played role in the detection of pathogen DNA in ticks collected from infected hosts.
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O60
Babesiosis in a Northern German cattle herd – epidemiological investigations

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Babesia divergens, transmitted by the tick *Ixodes ricinus*, is the most common cause of bovine babesiosis in Northern Europe and plays a role as a zoonotic pathogen in immunocompromised humans. In endemic areas, most cattle are immunologically protected from clinical disease in case they acquired the pathogen as a calf. Here, we report an unusually large outbreak of clinical babesiosis in an organic Northern German beef production farm located in a popular recreational area. In May 2018, 21 adult cattle died within a short period, showing classical symptoms of babesiosis. Remarkably, the majority of the affected animals had been raised on the farm, which had not previously experienced any babesiosis outbreak. This study aimed to investigate the local epidemiological situation by assessing *Babesia* prevalence in the farms" entire cattle stock as well as in the local tick population.
Blood smears of symptomatic animals were examined and presence of *B. divergens* was confirmed morphologically and by PCR amplification of a fragment of the 18S rRNA gene, followed by sequencing. In the following, blood samples of the entire remaining stock (N = 137) were tested for presence of *B. divergens* DNA by PCR. Furthermore, 493 adult and nymphal ticks were collected by the flagging method on two pastures, morphologically identified and subjected to *Babesia*-PCR and sequencing. *Babesia* DNA was still detected in eight animals of the affected herd despite prior treatment with imidocarb, whereas the remaining, untreated herds were negative in the *Babesia*-PCR. Overall, 10/493 ticks (2.0%) were *Babesia*-positive. Sequencing revealed presence of *B. microti* (7/493,

1.4%), *B. venatorum* (2/493, 0.4%) and *B. capreoli* (1/493, 0.2%). In contrast, *B. divergens* was not detected among the collected ticks.
The epidemiological data suggest a recent introduction of *B. divergens* onto one of the farm"s pastures. Since *B. divergens* was not found in adult and nymphal ticks, larvae might be implicated in transmission. In addition, two potentially zoonotic *Babesia* spp. were found in a popular recreational area.

O62

Typing of *Anaplasma phagocytophilum* strains by multilocus sequences typing (MLST), *ankA* gene sequencing and presence or absence of the *drhm* gene

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Introduction: *Anaplasma phagocytophilum* is a Gram-negative bacterium that is transmitted via tick-bite. It causes febrile disease in humans and domestic animals. Evidence for strain variation comes from cross-infection experiments showing that isolates from humans and animals differ in their pathogenicity for heterologous hosts. Further, it was suggested that in North America the presence of the *drhm* gene is indicative for strains not infectious for humans.
Objectives: Our goal was to explain the host preference of *A. phagocytophilum* strains by their molecular characterization using multilocus sequences typing (MLST), partial *ankA* gene sequencing and the absence or presence of the *drhm* gene.
Material & methods: 501 samples from 48 humans, 118 domestic animals, 94 farm animals, 145 large wild animals, 57 small mammals and 39 *Ixodes* spp. ticks were included. 480 originated from Europe and 21 from North America. 7 housekeeping genes were chosen for multilocus sequence typing (MLST). A partial *ankA* gene sequence was used for phylogenetic analysis. Further, the presence or absence of the *drhm* gene was determined.
Results: MLST and *ankA* gene-based typing revealed that European *A. phagocytophilum* strains from humans, dogs and horses were homologous, because they were part of the same clonal complex and the same *ankA* gene cluster. Most of the strains from North America belonged to a separate clonal complex containing sequences from humans and dogs. All North American strains lacked the *drhm* gene. However, with the exception of 3 samples from dogs, the *drhm* gene was present in all European strains from humans, dogs and horses. *A. phagocytophilum* strains from roe deer as well as from voles and shrews belonged to different MLST- and *ankA*-gene clusters than all other strains.
Conclusions: MLST and *ankA* gene sequencing are suitable for typing of *A. phagocytophilum* strains. In contrast, the presence of absence of the *drhm* gene was not appropriate to predict pathogenicity for humans.

POSTER ABSTRACTS

Lyme Borreliosis | LB-1 – LB-17

LB-1

Crystal structure of BB0365 from Lyme disease agent *Borrelia burgdorferi*

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Spirochete *Borrelia burgdorferi* is the causative agent of Lyme disease. Lyme disease spirochetes resides in the infected *Ixodes* ticks and are transferred to the mammalian host during the tick"s blood meal. Taking into account that the causative agent of Lyme disease resides either in the vector organism or mammalian host, several proteins have been described to be up/down-regulated according to the environmental factors. While there are several proteins known to be essential to cause the Lyme disease in mammals, the outer surface protein BB0365 has been identified to play important role for the persistence and survival of spirochetes in the feeding tick vector. We have determined the crystal structure of *B. burgdorferi* BB0365 at 2.1 Å resolution and made a comprehensive structural analysis to shed some light to the potential function of the protein. The structure of BB0365 undoubtedly helps to reveal the molecular details of the interaction between the mammalian host and an arthropod vector.

Acknowledgments
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LB-2

Tick-borne pathogens in *Ixodes ricinus* ticks from Ukrainian urban parks

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Hard ticks transmit a broad range of viral, bacterial and protozoan pathogens to humans and animals. The most abundant and epidemiologically the most important hard tick in Europe is *Ixodes ricinus.* It is the main vector of a wide spectrum of viral, bacterial and protozoan pathogens that causes *tick*-borne *encephalitis* (TBE), Lyme borreliosis, granulocytic anaplasmosis, spotted fever group rickettsiosis, tularemia and babesiosis (Rizzoli et al., 2014).
The aim of our study was to investigate the prevalence and genetic diversity of tick-borne pathogens in questing *I. ricinus* ticks collected in the Ukrainian urban parks.
In 2015 and 2016, altogether 1 058 *I. ricinus* ticks were collected by flagging the vegetation in 5 Ukrainian big cities: L"viv, Rivne, Zhytomyr, Kyiv and Poltava.
A total prevalence of tick-borne pathogens was 45.6%: 45.5% in L"viv, 31% in Rivne, 25.8% in Zhytomyr, 53.9% in Kyiv and 43% in Poltava urban parks.

Spirochetes from *B. burgdorferi* s.l. complex were found in 19% of examined ticks: 23% in L"viv, 17% in Rivne, 11% in Zhytomyr, 24% in Kyiv and 13% in Poltava. Seven genospecies were identified: *B. afzelii* (89%.), *B. garinii* (7%), *B. burgdorferi* s.s. (1.25%), *B. valaisiana* (1.25%), *B. bavariensis* (0.50%), *B. spielmanii* (0.50%) and *B. lusitaniae* (0.50%). *B. miyamotoi* was found in 1.5% of ticks. The phylogenetic relationship of spirochaete strains from Ukraine were studied with MLSA (based on 8 housekeeping genes).
The prevalence of infection with *A. phagocytophilum* was 5.6%. *Rickettsia* spp. was found in 17% of ticks: 99% *R. helvetica* and 1% *R. monacensis*. *Francisella tularensis* was not found in studied samples. A total of 4% *I. ricinus* were confirmed to contain *Babesia* spp.: 98% *Bab. microti* and 2% *Bab. venatorum*.
This study was financially supported by the National Scholarship Program for the Support of Mobility of Students, PhD students, Researchers and Artists (SAIA) in 2015-2016, project APVV 16-0463 and VEGA 2/0119/17.

LB-3

NMR structural analysis of Decorin binding protein A from *Borrelia afzelii*, the main factor of virulence

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Decorin binding protein A (DbpA) is a main factor of virulence of borrelia during the infection of the host. DbpA acts as an adhesin which binds glycosaminoglycan (GAG) chains of the extracellular matrix proteoglycan decorin. The previously determined structure of DbpA from *B. burgdorferi* proved its helical structure with two strain-specific variable parts important for GAG binding specificity. In this study, NMR structural analysis of DbpA from *Borrelia afzelii* was performed. 15N and 13C labelled recombinant DbpA was produced in a bacterial expression system, the protein was isolated by Ni2+ affinity chromatography with imidazole gradient elution and buffer exchange. Solution NMR spectra of the protein were measured on a 700 MHz spectrometer (Bruker) and assigned to 15N-HSQC and 15N TROSY-HSQC . Assigned spectra were HNCO, HNCA, CBCA(CO)NH and HNCACB for backbone assignment, CC(CO)NH, H(CCO)NH, 15N-TOCSY HSQC for sidechains and 15N-NOESY HSQC for the structure determination. The determined structure of the *B. afzelii* DbpA is compared to the known structure of DbpA from *B. burgdorferi* with an emphasis on variable parts.

LB-4
Genome assembly of the reptile-associated *Borrelia turcica*

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The genus *Borrelia* comprises arthropod-borne bacteria that are divided into three groups: the Lyme borreliosis (LB) group of spirochetes, the relapsing fever (RF) group and reptile-associated species (*Borrelia turcica*). Members of the latter group, although phylogenetically more similar to the Relapsing fever species, show some genetic similarity to LB species. They are transmitted by hard ticks and are of unknown human pathogenicity. The genome of *B. turcica* is typical for *Borreliae*. It"s type strain IST7 contains a linear chromosome (approx. 900 kb), a large linear plasmid (approx. 130 kb) and numerous short linear and circular plasmids (< 40bp). In this study, a comparison of *de novo* assemblies using different sequencing technologies and assemblers is presented. We sequenced two different passages (P3, P8) of a *B. turcica* isolate (GR16) obtained from a feeding hard tick *Hyalomma aegyptium* in Greece. Short read (Illumina) and long read (Oxford Nanopore) technologies and different *de novo* assemblers (e.g. SPAdes, metaSPAdes, Unicycler, Canu) were employed. The genome assembly was more challenging for P3 than P8, likely due to non-Borrelia DNA in the culture. Depending on the assembler, we observed incomplete genome reconstruction and loss of sequence information. MetaSPAdes emerged as a useful tool for assemblies of *Borrelia* isolated from feeding ticks, which – in spite of several *in vitro* passages – still contained vector and/or host DNA. Since reliable genome data are essential for comparative genomics and *Borrelia* genomes are complex, it is important to establish which method is preeminent for assembly of their genomes.

LB-5
Population Structure of *Borrelia turcica* from Greece and Turkey

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Borrelia turcica, a member of the reptile-associated *Borrelia* clade, is vectored by *Hyalomma aegyptium*. Hosts are tortoises *Testudo graeca*. *B. turcica* is known from several Southeastern European countries. Here we analyzed the population structure of *B. turcica* isolates from Greece and Turkey using multilocus sequence typing (MLST). Samples derived from Greece (near Serres) and from Turkey (near Izmir) (2017/2018). For phylogenetic and goeBurst analysis of *B. turcica* we used data from 15 Greek and 28 Turkish samples, respectively. A maximum-likelihood phylogenetic tree based on MLST sequence data confirmed that the samples were divergent from Lyme borreliosis or Relapsing fever associated species. Within the *B. turcica* clade, samples of different geographic origin (Greece, Turkey) clustered together in terminal branches; no differences between the Greek and Turkish samples were obvious.

A goeBURST diagram based on allelic profiles using triple locus variant (TLV) revealed very few clonal complexes with most samples appearing as singletons. Minor clonal complexes (two sequence types) contained only Greek isolates, only Turkish isolates or both. Furthermore, no signs of recombination was determined by comparing phylogenetic and goeBURST data. Thus, interestingly, very little population structure was discerned in our study. This was surprising in view of the large geographic distance between collection sites of *B. turcica* and raises questions about the mechanisms for maintaining independent lineages.

LB-6
Establishment and function of new cytokine ELISpots for the diagnosis of Lyme Disease

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Ticks are vectors of a great variety of pathogens. One of the most common vector borne illness is borreliosis (Lyme disease), a multi-systemic disease caused by *Borrelia burdorferi s.l.*, which is a gram-negative corkscrew-shaped spirochaete. In Germany, the probability to get Borreliosis after a tick bite is about 1%. The clinical manifestations of Lyme disease vary (Erythema migrans, Lyme neuroborreliosis, Lyme arthritis, Lyme carditis or Acrodermatitis chronica atrophicans) and although the symptoms are serious, currently there is no significant diagnostic method showing the requirement of treatment.
Therefore a new additional Borreliosis diagnostic tool, which is able to monitor the immune system, especially T cell cytokines which respond to the bacterium, must be developed to eliminate this deficiency. One option would be the T cell ELISpot. ELISpots represent very sensitive methods to detect single cytokine secreting cells of PBMCs, which are purified out of the blood. In TBC diagnostic, the Interferon gammy ELISpot is already a valid and standardized method.
Up to now, our team has established six new ELISpot protocols (IL-21, IL-22, IL-4, IL-6, IL-10 and IL-17 T cell ELISpot) and is currently testing seven Borrelia antigens on PBMCs of probands with and without Borreliosis in these new ELISpot systems in contrast to IFNg T ELISpot.
A discriminating ELISpot protocol based on the cytokines mentioned above to determine active Lyme disease has not succeeded yet, but many significant correlations among the produced cytokines of Borreliosis patients and those of healthy probands where found.
This results show new insight in the Borreliosis immune pathology in humans.

LB-7

Borrelia maritima sp. nov., a novel genospecies of the _B. burgdorferi_ sensu lato complex, occupies the most basal position in the North American clade

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Borrelia spp. are vector-borne parasitic bacteria with unusual, highly fragmented genomes that include a linear chromosome and linear as well as circular plasmids that differ numerically between and within various genospecies. *Borrelia* isolate CA690 was cultivated from a questing *Ixodes spinipalpis* nymph in the San Francisco Bay area, California, and was found to be genetically distinct from all other described genospecies belonging to the *Borrelia burgdorferi* sensu lato complex. We used Illumina MiSeq sequencing, and discovered that the similarity score of the main chromosome was ≤91% compared with other *Borrelia* spp. chromosomes. The genome including plasmids was assembled using a hybrid assembly of short Illumina reads and long reads obtained via Oxford Nanopore Technology. This work showed that the genome of CA690 consists of a main linear chromosome containing 902,176 bp and five linear and two circular plasmids. According to their PFam32 proteins they correspond to lp54, lp36, lp28-2, lp28-4, lp17, cp26, and cp32 in other species and showed partial similarity to these plasmids in various other *Borrelia* species. A phylogeny based on 37 single-copy genes of the main linear chromosome and rooted with the relapsing fever species *Borrelia duttonii* Ly revealed that CA690 had a sister-group relationship with, and occupied the most basal position of, the North American clade. We proposed the name *Borrelia maritima* sp. nov. for this novel genospecies as the type strain was isolated from a maritime-influenced climatic zone of California.

LB-8

High species diversity of Lyme disease spirochetes in _Ixodes ariadnae_ ticks collected from four _Myotis_ species bats in Poland

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The newly described *Ixodes ariadnae* Hornok 2014, is a cave-dwelling hard tick species associated with European bats mostly from the genus *Myotis,* and with *Plecotus auritus*.

This long-legged tick which is morphologically very similar to *I. vespertilionis*, has been so far reported from Hungary, Germany and Belgium. To our knowledge, its role as a potential vector or reservoir of bacterial agents has not been studied, and therefore is completely unknown. The main goal of this research was to determine if *I. ariadnae* ticks feeding upon four *Myotis* species may be infected with *Borrelia burgdorferi* s.l. The sampling was conducted in six caves of the Carpathian Mountains in October/December 2011 and in February/March 2012. Engorged long-legged ixodid ticks derived from 30 bats belonging to four *Myotis* species: *M. myotis* (n=27), *M. daubentonii* (n=1), *M. emarginatus* (n=1), *M. mystacinus* (n=1). Ticks were identified morphologically and molecularly by the cytochrome oxidase subunit I (COI) gene analysis. A total of 62 *I. ariadnae* individuals (4 larvae, 22 nymphs, 36 females) were screened individually for the presence of *Borrelia* DNA by using a nested PCR assay based on *flaB* gene. *Borrelia* species identification was conducted with PCR-RFLP using HpyF3I and EcI136II restriction enzymes and partial *flaB* gene sequencing. *Borrelia* DNA was detected in 27 (43.5%) of 62 ticks. PCR positive ticks were obtained from all *Myotis* species included in this research. Comparable infection rates were found in nymphs (41%) and females (42%). In total, seven spirochete species from the *B. burgdorferi* s.l. complex were identified among infected ticks. *B. spielmanii* (33%) and *B. carolinensis* (30%) were the most prevalent, followed by *B. afzelii* (15%), *B. burgdoferi* s.s. (11%), *B. garinii, B. valaisiana*, and *B. lanei*. The study demonstrates for the first time the presence of *B. burgdorferi* s.l. infection in nidicolous *I. ariadnae* ticks specific to bats. This non-human-biting tick seems to participate in silent enzootic transmission cycles for certain LB spirochetes acting as their potential vector.

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LB-10

Usefulness of biological samples for *Borrelia burgdorferi* s.l. infection status assessment in avian hosts

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Avian hosts can act as reservoirs for *Borrelia burgdorferi* sensu lato (s.l.), a bacterial complex that includes the etiologic agents of Lyme borreliosis. It is relevant to evaluate the infection status of wild bird species to elucidate their importance as reservoir hosts, because not all contribute equally to the maintenance of *Borrelia.* There is a lack of a reliable non-invasive technique that allows us to assess the infection status of *B. burgdorferi* s.l. in wildlife. Thus, the main goal of this study was to evaluate the usefulness of biological samples such as blood and skin biopsies from two avian model species, *Turdus merula* and *Erithacus rubecula,* to diagnose *B. burgdorferi* s.l. infection and give information on their infectivity to ticks.

Blood and skin tissues were collected from 16 *Erithacus rubecula* and 10 *Turdus merula* captured in *Borrelia* enzootic areas. These birds were taken into captivity and subjected to xenodiagnoses. DNA was extracted from blood and skin samples and from the fed xenodiagnostic larvae, and analysed for *B. burgdorferi* s.l. infection by real-time PCR directed to the *flaB* gene.

Sensitivity of these approaches to evaluate host"s infection status will be compared and will help to elucidate *B. burgdorferi* s.l tropism in the birds" organism.

LB-11
Cervical myelitis as uncommon manifestation of neuroborreliosis – a case report

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Introduction: Besides the most frequent clinical manifestations of neuroborreliosis, some are atypical, rare and may be the reason of the diagnosis and treatment delay.
The aim of the study is the presentation of the case of cervical myelitis in the course of neuroborreliosis. Case report: A 23-year-old female was admitted to the Department of Neurology and consulted at the Clinical Department of Infectious Diseases in September 2018 due to intensive tremors and paresthesia of the upper extremities as well as cervical spine pain. Anamnaesis: potential exposures to tick bites in early May 2018, followed by fever states, headache, nausea and anorexia lasting from the end of May to September, with periodic intensification and remissions. The above symptoms, in outpatient diagnostics, were attributed to viral infection, migraine, psychical disorders, thyroid gland disease and suspicion of chemical intoxication.
Results: MRI(outpatient-14.09.18): in the cervical section from C1/C2 to C5/C6 an abnormal region of elevated signal in T2 and STIR images within the middle and back of the spinal cord. Areas of very poorly expressed enhancement after i.v. administration of the contrast agent. The patient reported to the hospital only after 2 weeks. MRI(27.09.18):A large degree of distention of the cervical cord almost all the length with incorrect signals. The results of laboratory tests were as follows: serum-CLIA:IgM-92AU/ml, IgG-239AU/ml;Western blot:IgM–positive, IgG–positive; antyVlsE/C6 IgG–1462,0 RU/ml; cerebrospinal fluid (CSF): pleocytosis 323/μL,70% lymph., protein-2,15g/L, B.b. CSF/serum antibodies index (AI)>1,5csq-positive. Ceftriaxone i.v. (28-day) treatment resulted in the almost complete withdrawal of symptoms (with residue of trace, periodic tremors of hands) and evident improvement of the patient"s clinical state.
Conclusion: *Borrelia* b. s.l. infections should be taken into consideration in a wide aspect of neurological disorders differential diagnostics.

LB-12
Molecular survey of tick-borne encephalitis virus and *Borrelia* diversity in *Ixodes ricinus* ticks from natural habitats in North East Germany

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Ixodid ticks transmit the greatest variety of pathogens when compared to other arthropods. From the almost 900 species of ticks worldwide, *Ixodes ricinus* is the most widespread species in Europe and

Germany. It is known to feed on many different hosts and is capable to transmit numerous pathogens. Most relevant pathogens for which *I. ricinus* is a competent vector are *Borrelia burgdorferi* sensu latu, the agent responsible for Lyme disease and the tick-borne encephalitis virus.
The aim of this study was to assess the prevalence rates with molecular methods of TBEv and *Borrelia* species in *I. ricinus* ticks from North East Germany.
In order to perform the study, we collected ticks by flagging from 18 forest sites in Western Pomerania between April and October 2018. Samples were processed by RNA and DNA extractions, performed from each individual adult tick and from pools of 10 nymphs. RNA samples were tested by RT-qPCR for detection of TBEv while DNA was tested by nested PCR followed by sequencing for identification of *Borrelia* species. A total of 2619 ticks were collected of which 244 were females, 249 males and 2126 nymphs.
So far, after analyzing 249 samples for *Borrelia* spp., 61 (24.5%) ticks tested positive. The comparison between developmental stages showed a higher prevalence rate in nymphs (34.8% vs 23.9% of females vs 14.6% of males). Sequencing revealed several *Borrelia* species relevant for public health: *B. garinii, B. afzelii, B. valaisiana* and relapsing fever agent *B. miyamotoi*. RT-qPCR for TBEv is undergoing, no positive samples being detected until now.
Our findings are in concordance with previous reports indicating *Borrelia* spp. as the most prevalent tick-borne pathogen in Northern Hemisphere. Extensive future studies should determine the natural foci of TBEv in Western Pomerania.

LB-13
Longitudinal study of infection with *Borrelia* spp. in questing *Ixodes ricinus* from northwestern Spain

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Introduction: *Borrelia burgdorferi* sensu lato (s.l.) is the most prevalent tick-borne pathogen in Europe where it is transmitted by *Ixodes ricinus*; this tick also circulates *Borrelia miyamotoi*, a member of the relapsing-fever group.
Objectives: The objective of this study was to assess the prevalence and genetic diversity of *Borrelia* spp. in 1,056 *I. ricinus* from north-western Spain. The effect of some variables (year, area and stage of development) on the prevalence of *Borrelia* spp. in ticks was assessed. The periods of acarological risk were established after analysis of the distribution of the percentage of *Borrelia*-infected ticks throughout the study.
Materials & methods: Ticks were monthly collected by flagging in three different ecological areas from north-western Spain during two years. *Borrelia* DNA was detected by a PCR targeting the *fla* gene; positive samples were also characterized at the *IGS* region and the *GlpQ* gene.

Results: *Borrelia burgdorferi* s.l. DNA was detected in the 11.84% of *I. ricinus*, and five genospecies were identified (*Borrelia afzelii, Borrelia burgdorferi sensu stricto, Borrelia garinii, Borrelia lusitaniae* and *Borrelia valaisiana*). *Borrelia miyamotoi* DNA was also found (0.85%). Mixed *B. burgdorferi* s.l.-*B. miyamotoi* infections were also detected (0.38%). The prevalence of *B. burgdorferi* s.l. was significantly higher in female ticks and in the mountain where the highest altitude and lowest average annual temperatures were recorded. In addition, a temporal pattern in the *B. burgdorferi* s.l. prevalence distribution throughout the study was not detected and no correlation between the total number of ticks captured and *Borrelia burgdorferi* s.l. prevalence was found. Therefore, the acarological risk for acquiring LB may increase with the density of questing ticks.
Conclusion: The detection of a noticeable prevalence of *B. burgdorferi* s.l. in questing ticks suggests a high acarological risk, especially in the mountain area.

LB-14
Determination of the complement-inhibitory activity of different outer surface proteins of *Borrelia recurrentis*

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Introduction: *Borrelia recurrentis* is the only causative agent of the Louse-borne relapsing fever (LBRF) and claimed as a "neglected arthropod-borne pathogen". One strategy of spirochetes to evade innate immunity involves binding of complement regulators or complement components. Two complement-interacting surface proteins, CihC and HcpA have been characterized so far in *B. recurrentis* but a cluster of nine additional genes encoding for HcpA-homologs is localized adjacent to the *hcpA* gene on the same linear plasmid.
Objectives: The goal of this study is aimed at identifying novel determinants for immune evasion that act as potential pathogenicity factors of *B. recurrentis*.
Material & methods: An ELISA-based approach was chosen to examine the inhibitory potential of the recombinant proteins generated on the alternative (AP), classical (CP) or lectin pathway (LP). In addition, hemolytic assays were employed to further assess the inactivation capacity of the borrelial proteins on the CP and the terminal pathway (TP). Further the binding properties of each borrelial protein to Factor H, Factor I, C3b, and C5 were analyzed by ELISA. Finally, the inhibitory effect on the TP was investigated by examining the polymerization of complement component C9.
Results: Initially, we functionally characterized four out of nine proteins, all of which specifically inhibited the AP but not the CP or LP. All four proteins examined bound C3b in a dose dependent manner but not Factor H, Factor I, and C5. These proteins also inhibited the TP and blocked the polymerization of C9 dose-dependently.
Conclusion: Taken together, the data collected suggest that all proteins analyzed were able to specifically inhibit the AP probably through the interaction with C3b. Furthermore, three proteins inhibited the polymerization of C9 and thereby counteracted the assembly of the TCC.
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LB-15

Prevelance and genetic classification of*Borrelia Burgdorferi* Sensu Lato in different study sites in Slovakia

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Lyme borreliosis is the most prevalent tick-borne disease in Europe. Genetic variability within and between the *Borrelia burgdorferi* s.l. genospecies is linked to different clinical symptoms as well as their different association to reservoir hosts. The aim of study was to examine the prevalence, genospecies distribution and genetic classification of *B. burgdorferi* s.l. at different sites in Slovakia. Questing ticks were collected in Slovakia (urban park in Malacky and Bratislava, mixed forest Martinské hole Mountains and suburban forest in Košice) and were tested for *Borrelia*-prevalence by amplification of 222-255 bp fragment of 5S-23S rRNA intergenic spacer. Identification of *Borrelia* genospecies in positive samples was further conducted by RFLP assay. Genetic diversity between and within genospecies was assesed by Multilocus sequence typing (MLST).
Prevalence of *B.b.* s.l. warried from 16% in Bratislava to 41% in Košice. The most abundant genospecies were *B.afzelii* (Bratislava, Malacky 54.5% and 43% respectively), *B.garinii* (Košice, 30%) and *B.lusitaniae* (Martinske hole, 45.5%). Multilocus sequence analysis revealed new allelic profiles of *B.afzelii, B.garinii, B.lusitaniae* and *B.spielmanii*. *B.lusitaniae* from Martinské hole were genetically more closely related to Serbian isolates than to the Portuguese strains of *B. lusitaniae.* Two samples were represented by unique sequence type.
The results of this study proved usability and effectivity of MLST method for studying genetic variability and phylogeny of *B. burgdorferi* s.l.
Acknowledgement: This study was financially supported by Project of VEGA No. 2/0119/17 and by Project of Slovak Research and Development Agency APVV 16-0463.

LB-16

Prioritizing Lyme borreliosis risk areas for forest and nature management based on novel insights in tick ecology

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Occurrence of tick-borne diseases in humans is driven by tick population density, pathogen prevalence and contact rate between ticks and humans. Variation in tick densities and pathogen prevalence between forests is well studied, but much less is known about spatial distribution within forest parcels despite its importance in management of individual forests.

We present an approach integrating drag sampling of ticks and host camera trapping on fine scale plots, divided in sub-plots of 4X5 m^2 at (1) a structural element, (2) 40 meters further along the adjacent trail, (3) in the interior of the forest, (4) at a playing area and (5) along a forest edge trail. This will allow us to study the local variability in tick density and pathogen prevalence with respect to forest structure and recreational infrastructure. It also enables the analysis of relations between density of infected nymphs, visitor flows and host habitat use. We will test how host habitat use relates to forest stand type, recreation infrastructure and human disturbance and in turn will have an effect on tick abundances and the prevalence of pathogens. A pilot study was conducted in spring and summer 2018, testing the drag sampling design in 36 triplets (1-3), divided over 10 forests. The camera trapping approach was tested in two triplets. Significantly more ticks were captured in the forest interior ($P < 0.001$) and adjacent to trails ($P = 0.002$) compared to sites at structural elements. The application of this approach on a larger scale, within a four-year PhD project, will enable the designation of priority locations for tick population management.

LB-17

Detection of *Borrelia burgdorferi* sensu lato in a recently established population of the taiga tick, *Ixodes persulcatus* in Sweden

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The tick species *Ixodes ricinus* and *Ixodes persulcatus* are the main vectors of Lyme borreliosis spirochetes in the western and eastern parts, respectively, of the Palearctic region. In parts of Russia and Finland *I. persulcatus* has increased its range and abundance. Recently permanent populations of the taiga tick, *I. persulcatus* were detected for the first time in northern Sweden. This prompted us to investigate the potential occurrence of different genospecies of Lyme borreliosis spirochetes in the taiga tick in northern Sweden.

In May-July 2016 *I. persulcatus* ticks were collected by the cloth-dragging method at five mixed woodland, island localities in the Bothnian Bay, province of Norrbotten, northern Sweden. Ticks were microscopically and molecularly identified to developmental stage and species and screened for *Borrelia* spp. using quantitative PCR. The *Borrelia* genospecies composition of the quantitative PCR-positive samples was determined by conventional PCR followed by sequencing.

A total of 266 *I. persulcatus* ticks [134 adult males (50%), 127 adult females (48%), and 5 nymphs (2%)] were collected and screened for the presence of *Borrelia* spp. Overall, 58% of the ticks (155 of 266) contained *Borrelia* spp. There were no significant differences in prevalence between developmental stages or sex of adult ticks (adult males, 57%; adult females, 58%; nymphs 80%). Three *Borrelia* species were identified by sequence analysis. *B. afzelii* was the predominant species and was detected in 46% of all ticks containing *Borrelia*, followed by *B. garinii* (33%), *B. valaisiana* (1%), and mixed infection of *Borrelia* species (1%); 19% could not be identified to species.

Here we report for the first time the presence of Lyme borreliosis spirochetes in the taiga tick collected from Sweden. Our results suggest a twofold higher prevalence of *Borrelia* spp. in this *I. persulcatus* population compared to *I. ricinus* from more southern regions of Sweden.

LB-19

Altered gene expression upon infection with *Borrelia afzelii* in nymphal *Ixodes ricinus* salivary glands during feeding

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Ixodes ricinus is the vector for a variety of pathogens that greatly affect human health, including *Borrelia afzelii*, the dominant causative agent of Lyme borreliosis in Europe. The relationship between *I. ricinus* and *Borrelia* is dynamic; transcription of specific tick genes is known to be changed in the presence of *Borrelia*. Especially during transmission of *Borrelia burgdorferi*, specific changes in tick gene expression have shown to be vital for successful infection of the vertebrate host. However, it is thus far not known if and to what extend *Borrelia afzelii* influences gene expression in tick salivary glands. In the current study we measured expression of tick salivary gland proteins (TSGPs) during tick feeding. Large and small RNA was isolated from pooled tick salivary glands (TSG) from unfed, 24 hours feeding and fully engorged *B. afzelii*-(un)infected *I. ricinus* nymphs and used for RNAseq and Massive analysis of cDNA ends (MACE). MACE was used to quantify tick gene expression. MACE reads were mapped against contigs from our own RNAseq data supplemented with contigs from several bioprojects and subsequently annotated against Swissprot, Trembl or NCBI databases successively, resulting in the identification of 26.179 tick transcripts. We showed that tick feeding is the main expression differentiator; yet *Borrelia* infection significantly differentiated expression of transcripts in uninfected TSGs, at 24 hours after onset of feeding and in fully fed nymphal TSGs. Our studies will contribute to a better understanding of the dynamic interplay between *I. ricinus* and *B. afzelii* and will reveal new insight into early Lyme borreliosis pathogenesis.

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POSTER ABSTRACTS
Ticks & others | T&O-1 – T&O-39

T&O-1
Expansion and control of the tick *Dermacentor reticulatus* in Poland

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Question: Dynamic expansion of *Dermacentor reticulatus* ticks in many areas in central Europe, including western and central Poland, generated a lot of problems, including the spread of canine babesiosis and *Rickettsia* pathogens. During last six years we monitored tick expansion, host usage and vector role.
Methods: We studied the influence of agricultural practices on the densities of tick in open areas (on pastures, managed meadows and fallow lands). In two long-term field experiments, we determined the role of regular mowing and cessation of mowing on the densities of ticks. Finally, we determined the impact of spring burning of grasses on tick abundance.
Results: We discovered that *D. reticulatus* constituted the dominant tick species (80%) on livestock and dogs, especially in spring and autumn. We confirmed its continuous expansion, covering up to 50 km in year. Tick infestation was found in rodents and red foxes from newly inhabited areas. We confirmed its role as a vector of TBEV, *Babesia canis* and *Rickettsia raoulti*. We discovered that tick densities were the highest in fallow lands and the lowest on permanently grazed pastures. Cessation of mowing caused continuous increase in tick abundance on newly created fallow lands. On the other hand, regular mowing and burning of grasses in spring resulted with significant (several time) decrease in tick densities.
Conclusions: Cessation of agricultural practices may have facilitated the spread of the tick but regular mowing may be implemented as effective control measure for tick population and tick-borne diseases.
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T&O-2
The Tick Cell Biobank – new developments and associated research

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Introduction: Tick cell lines (TCL) are increasingly used in many fields of tick and tick-borne disease research, including isolation, propagation and study of arboviruses and bacteria, tick biology and tick control.

Objectives: The Tick Cell Biobank (TCB) was established almost a decade ago to facilitate the development and uptake of these unique and valuable resources by the global tick research community.
Materials & methods: As well as being a repository for existing and new ixodid and argasid TCL, the TCB supplies TCL and training in their maintenance to scientists worldwide and generates novel cell lines from tick and other arthropod species (midges, sandflies, tsetse flies, fish lice) not already in the collection. Studies are under way to sequence the genomes of selected TCL, to detect, isolate and characterise endosymbiotic bacteria harboured by ticks, and to develop protocols for transcriptomic and proteomic analysis of tick-virus interactions.
Results: The TCB now houses >60 arthropod cell lines, many of which have been distributed to ~80 institutes worldwide, and has trained >90 young scientists from Europe, Asia, Africa and the Americas. TCB Outposts are being set up in Malaysia, Kenya and Brazil to facilitate uptake and exploitation of TCL and associated training by scientists in these and neighbouring countries. Several Rickettsia and Spiroplasma isolates have been added to the TCB"s collection of intracellular bacteria. In-house and collaborative studies have revealed the presence of integrated bacterial and viral genetic elements in some TCL genomes, developed in vitro culture systems for previously uncultivable bacteria and elucidated aspects of tick-virus interactions.
Conclusions: The TCB has contributed to an exponential rise in the amount and quality of tick-related research outputs within Europe and worldwide, and will to continue to underpin global research into biology and control of ticks and tick-borne diseases for many years to come.

T&O-3
New records of *Compluriscutula vetulum* larvae in Burmese amber, with notes on morphology

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The first fossil assignment to the extinct hard tick *Compluriscutula vetulum* (Ixodida: Ixodidae) by Poinar and Buckley in 2008 was based on a larva. Here, four more larvae of this species are described, with notes on morphological features based on a Keyence microscope 6000 and Rontgen microCT images. All four specimens present almost the same morphological characters. The only significant difference is the presence of 14 festoons and not 13 as in the previous description. These results show that *C. vetulum* was fairly common in the mid-Cretaceous (ca. 100 Ma) Burmese amber of Myanmar. So far, only the larvae are known and it seems that they preferred arboricolous hosts.

T&O-4
High numbers of *Hyalomma* ticks in Germany 2018

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Hyalomma marginatum and *Hyalomma rufipes* are two-host tick species, which are mainly distributed in southern Europe, Africa and middle-eastern Asia. They are well-known vectors of Crimean Congo hemorrhagic fever (CCHF) virus and other viruses as well as *Rickettsia aeschlimannii*. In recent years, these tick species have been found sporadically in Germany, but they do not belong to the autochthonous tick fauna in Germany.
Ticks with unusual morphology were collected and sent from private persons or public health offices to involve institutions for morphological identification and further testing. All ticks identified as *Hyalomma* spp. were tested using molecular detection methods for CCHF virus, *Rickettsia* spp., *Coxiella burnetii* and *Coxiella*-like organisms, *Babesia* spp. and *Theileria* spp.
Thirty five ticks with unusual appearance or behaviour were reported during summer-autumn 2018 to us. For 17 of them description or photos imply that they were belonging to the hard tick genus *Hyalomma*. A total of 18 ticks were sent to us and could be identified as adult *Hyalomma marginatum* (10 specimens) or adult *Hyalomma rufipes* (8 specimens). All ticks tested negative for CCHF virus, *Coxiella burnetii*, *Coxiella*-like organisms, *Babesia* spp. and *Theileria* spp. The screening for rickettsiae gave positive results in nine specimens. The *Rickettsia* species in all cases was identified as *R. aeschlimannii*. These results show that exotic tick species are imported into Germany and were able to develop from the nymphal to the adult stage under appropriate weather conditions. Fifty percent of the ticks carried *R. aeschlimannii*, a human pathogen, while CCHF virus or other pathogens were not detected. Imported *Hyalomma* ticks may be the source of exotic diseases acquired in Germany.

T&O-5
Ixodes ricinus salivary serpin IRS-1 as a modulator of the host immune response

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Introduction: Ticks secrete a wide range of bioactive molecules via saliva into their host. Serpins represent one group of secreted salivary protease inhibitors used by ticks to counteract host haemostatic and/or immune response to blood feeding.

To date, a specific function has been ascribed only to two serpins from the tick *Ixodes ricinus* – a major European vector of several medically important pathogens.
Objectives: The aim of this research was to functionally characterize another *I. ricinus* serpin IRS-1 (*I. ricinus* serpin 1), with an emphasis on its capacity to modulate the immune response of the host.
Materials & methods: The expression of IRS-1 in nymphs and adult females' salivary glands, midguts, and ovaries was assessed using the quantitative real-time PCR. The effect of IRS-1 on neutrophil, monocyte, and eosinophil migration was evaluated *in vitro* and *in vivo* by using the transwell chambers and mouse model of sterile peritonitis induced by the intraperitoneal administration of thioglycollate. Moreover, the ability of IRS-1 to interfere with the production of selected cytokines and chemokines was tested *in vitro* in unstimulated and lipopolysaccharide-stimulated resident peritoneal cells as well as *in vivo* in thioglycollate-induced peritonitis.
Results: IRS-1 exhibited an immunomodulatory effect since it inhibited the migration of neutrophils and monocytes, while not affecting the recruitment of eosinophils. IRS-1 also altered the production of several cytokines and chemokines, some of which are associated with Th2 immunity.
Conclusion: IRS-1 might facilitate tick engorgement by modulating the host immune response to blood feeding.

T&O-7
The Ixogon® Zeckenrollen - A field trial to test the efficacy of a tick control system

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Over the last years, studies by the University of Hohenheim have shown that gardens play an important role as tick habitat. In order to specifically target ticks in gardens, the product Ixogon® Zeckenrollen was released in 2011. It consists of biodegradable cardboard tubes, filled with cotton which was treated with an acaricide. The concept is based on the expectation that small rodents living in the gardens will use the impregnated cotton as nesting material. Ticks attempting to take a blood meal on those rodents should die before finishing the meal as a result of the acaricide. To test the efficacy of the product as a tick control agent, a total of 20 gardens in Stuttgart and its surrounding area were chosen in spring 2016. Ixogon® Zeckenrollen were placed in ten of these gardens, the test gardens, while tubes with untreated cotton were placed in the ten control gardens. During the years 2016, 2017 and 2018, ticks were collected monthly in all gardens using the flagging method. Additionally, life traps were used to capture rodents, which were tested for traces of the acaricide. According to the current data, Ixogon® Zeckenrollen are capable of reducing the tick density up to 33 % in two years. The rodents generally accepted the nesting material provided by the Ixogon® Zeckenrollen. Furthermore, the acaricide could be detected on 64 % of the rodents caught in test gardens. Our results show, that Ixogon® Zeckenrollen can significantly reduce tick densities in gardens.

T&O-8

New finding of *Haemaphysalis concinna* in Western Poland

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Introduction: Relict tick *Haemaphysalis concinna* is the species of fragmented and focal distribution in Central Europe and Asia. Although in majority of neighboring countries the occurrence of this tick species is well-documented (i.e in Germany, Czech Republic, Slovakia, Ukraine), in Poland its occurrence was registered only once, in 1953 in Troszyn in Western Pomerania, close to German-Polish border.
Objectives: In present study we describe new finding of *H. concinna* in Western Poland, confirmed both by collection of juvenile ticks from rodent hosts and by collection of questing ticks from vegetation.
Materials & methods: Trapping sessions of rodents took place in the summer of 2018 in three locations in Western Poland. Trapped rodents were measured and weighed, their sex was determined. The ectoparasites were carefully removed during the inspection of fur, with particular consideration of pinnae. All collected ticks were assigned to species and stages according to morphological keys. Molecular methods were applied for genotyping of ticks.
Results: A total of 106 rodents were examined for the ectoparasites. Common tick *Ixodes ricinus* was found abundant on small rodents at all sites; *Dermacentor reticulatus* was identified at two sites in small numbers and, finally, numerous juvenile *H. concinna* were found at one site (Nowy Młyn 2). On one root vole *Microtus oeconomus* from this area altogether 273 ticks were found, including 115 *I. ricinus* and 158 *H. concinna*. Additionally, questing nymphs and adult *H. concinna* were collected from vegetation at this site (n=20). Genotyping and phylogenetic analysis confirmed species identification of *H. concinna*.
Conclusion: A new focus of *H. concinna* was described in Western Poland. Our long-term monitoring of *D. reticulatus* expansion in Poland suggest that *H. concinna* is still very rare in the area of Poland.
The study was financially supported by National Science Centre (NCN) Sonata Bis grant no. 2014/14/E/NZ7/00153.

T&O-9

Study of the life cycle of *Hyalomma excavatum* and *Hyalomma scupense* under laboratory conditions

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Introduction: In Tunisia, Tropical theileriosis (*T. annulata* infection) is a dominant infection causing important losses in the cattle industry. Several tick species of *Hyalomma* genus could transmit T. *annulata* experimentally. However, under natural conditions this transmission occurs with few tick species of monotropic behaviour.

In Tunisia, *Hyalomma scupense* is assuring this role. *Hyalomma* laboratory colonies are required to generate biological material for studying different aspects of tropical theileriosis.
Objectives: The objective of the work is to generate data on the chronology of the life cycle of two *Hyalomma* species maintained in colonies under laboratory conditions, namely *H. excavatum* and the natural vector of *T. annulata*, *H. scupense*.
Materials & methods: Adults were fed on cattle, *H. scupense* immatures instars were fed on rabbit. For *H. excavatum*, larvae were engorged either on rabbits to obtain a two hosts cycle, or on the gerbil *Meriones unguiculatus* if a three hosts cycle is needed. Engorged ticks of both species were incubated at 28°C and 85% Relative Humidity. A regular check was carried out to determine development and reproduction parameters.
Results: The mean duration of the entire life cycle is 109 and 130 days for *H. excavatum* and *H. scupense,* respectively. There was a positive correlation between tick weight and number of eggs produced for both species ($P<0.05$).
Conclusion: The present study provided useful information which contribute to understand better the biology of these species under natural conditions, to adjust control options used under field conditions and to improve logistical aspects regarding the production of biological material required for research work on tropical theileriosis.

T&O-10

Factors affecting co-feeding of *Ixodes* spp. ticks on rodent hosts in the Netherlands, an emerging area for TBEV

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Introduction: Tick-borne encephalitis virus (TBEV) is a growing public health concern in Europe. The virus was recently detected in questing *Ixodes ricinus* ticks in the Netherlands, and the first autochthonous human cases followed shortly after. Co-feeding between infected nymphs and uninfected larvae is considered to be the main transmission route of TBEV. Hence, identifying the factors that promote co-feeding aggregation is essential for understanding TBEV transmission dynamics.
Objectives: In this study, we aimed to quantify whether and how co-feeding aggregations on rodent hosts in the Netherlands can be explained by both extrinsic factors (i.e., temperature, vegetation type) and intrinsic factors (i.e., rodent species, sex, and body mass).
Materials & methods: Rodents were trapped on a weekly basis in five different forest habitats around Wageningen, the Netherlands, between March – May 2018. Rodents were sexed, weighted, checked for ticks, and released again. At each forest habitat, the mean temperature was recorded at a height of 50 cm.
Results: Using generalized linear models, we found that co-feeding aggregations were more common on wood mice (*Apodemus sylvaticus*) than on bank voles (*Myodes glareolus*), more common on males than females, and more common in mixed forests than in oak or beech forests. Co-feeding increased with rodent weight and cumulative mean daily temperature. Further, rodents parasitized by nymphs had higher larval burdens than rodents that did not carry nymphs.

Conclusion: Our study shows that co-feeding between nymphal and larval *Ixodes* spp. is common in Dutch rodents, particularly in male wood mice of high body mass in mixed forest habitats, and that co-feeding aggregation increases with cumulative mean daily temperature from early to late spring.

T&O-12
Two year monitoring of tick abundance in the city of Hanover

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Questing ticks may transmit a variety of human and animal pathogens. During the last 10 years, prevalence of *Borrelia* spp., *Rickettsia* spp. and *Anaplasma phagocytophilum* in Hanoverian ticks has been monitored. However, to determine the infection risk for humans and animals, not only pathogen prevalence but also tick abundance needs to be taken into account. Therefore, the tick abundance in the urban area of Hanover was quantified on a monthly basis from April to October in 2017 and 2018. In the first and second half of each month, questing ticks were collected at 10 different sampling sites, comprising 3 urban parks, 3 mixed forest and 4 broad-leaved forest areas, in the city of Hanover by the flagging method. A total area of 200 m^2 (divided into four 50 m^2 areas) was sampled at each sampling site. One of the four 50 m^2 areas was sampled on a rotational basis at each visit, resulting in 100 m^2 sampled per month. In addition, environmental factors of the sampling area were determined. Tick species differentiation and determination of the developmental stage was based on morphological characteristics.
In 2017, a total of 1,770 ticks were collected, while 1,866 ticks were collected in 2018. In 2017 and 2018 morphological species differentiation revealed 1630 and 1717 ticks as *I. ricinus*, 25 and 27 ticks as *I. inopinatus* as well as 3 and 18 ticks as *I. frontalis*. The remaining 216 ticks could not be clearly assigned to any species yet. Monthly tick density at the different sites ranged from 0 to 167 ticks/100 m^2 in 2017, with an average of 25 ticks/100 m^2, and from 0 to 223 ticks/100m^2 in 2018 (average: 27 ticks/100m^2). No significant difference was found regarding tick density between the two study years. Regarding different landscape types, ticks were most abundant in mixed forests, with more than 50 ticks/100m^2 on average in both years, whereas in broad-leaved forests an average of 13 and 19 ticks/100 m^2 was collected in 2017 and 2018, respectively. In urban parks, average tick density was 15 ticks/100 m^2 in 2017 and 11 ticks/100 m^2 in 2018. In both years, tick densities showed a marked peak in May and June at most sites whereas a less pronounced peak was recognizable in September.
To gain a more profound understanding of influencing factors and to obtain reliable data on tick density in the city of Hanover, the study will be continued in 2019.

T&O-13

Occurrence of *Ixodes inopinatus* in Northern Germany and prevalence of tick-borne pathogens

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The recently described tick species *Ixodes inopinatus* is closely related to *I. ricinus* and can be morphologically distinguished by subtle characteristics only. Occurrence of *I. inopinatus* was reported in southern Germany, western Austria and Romania whereas no data is yet available on its occurrence in northern Germany. Furthermore, the role of *I. inopinatus* in pathogen transmission is still unknown. Therefore, 3845 DNA samples from *Ixodes* ticks collected for prevalence studies on *Borrelia*, *Rickettsia* and *Anaplasma phagocytophilum* during the years 2010-2015 in Hamburg and Hannover were differentiated into *I. ricinus* or *I. inopinatus* by sequencing of a 16S-rRNA gene fragment.
In total, 3.56% (137/3845) of the sequenced ticks were assigned to the species *I. inopinatus*. Prevalence was 33.58% (46/137) for *Borrelia* spp., 45.99% (63/137) for *Rickettsia* spp. and 2.92% (4/137) for *A. phagocytophilum*. Thereof, adult males showed an detection rate for *Borrelia*, *Rickettsia* and *A. phagocytophilum* of 58.82% (10/17), 64.71% (11/17) and 5.88% (1/17), adult females of 50.00% (12/24), 58.33% (11/24) and 4.17% (1/24), and nymphs of 25.00% (24/96), 39.58% (38/96) and 2.08% (2/96). Detection rates in *I. ricinus* amounted to 24.78% (919/3708) for *Borrelia* spp., 46.63% (1729/3708) for *Rickettsia* spp. and 3.64% (135/3708) for *A. phagocytophilum*. By tick stages, 27.99% (110/393), 46.82% (184/393) and 4.33% (17/393) of adult males, 35.40% (143/404), 48.27% (195/404) and 7.67% (31/404) of adult females as well as 22.88% (666/2911), 45.69% (1330/2911) and 2.99% (87/2911) of nymphs were infected with *Borrelia*, *Rickettsia* and *A. phagocytophilum*. A coinfection rate of 21.17% (29/137) in *I. inopinatus* compared to 14.13% (524/3708) in *I. ricinus* was determined.
To the best of our knowledge, this is the first report of *I. inopinatus* in Northern Germany. Detection of DNA in the questing ticks indicates a potential role of *I. inopinatus* as a vector for *Borrelia*, *Rickettsia* and *A. phagocytophilum*.

T&O-14

Analysis of skin surface decontamination methods to assess unbiased tick-borne microbiomes

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Various viruses, bacteria, eukaryotes and pathogens have been found in *Ixodes ricinus,* a common tick in central Europe. However, most studies assess microbial communities of ticks without prior decontamination of the tick skin surface, which may alter the results and mislead tick-borne conclusions.

The aim of this study was to test four different skin decontamination methods namely i.) 70% Ethanol, ii.) DNA away, iii.) 5 % NaOCl and iv.) Reactive-skin-decontamination-solution (RSDL) that were previously reported for tick, surface, animal or human skin decontamination. To test efficiency of decontamination, we contaminated each tick with a defined mixture of *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens*, Cocksfoot mild mosaic virus (*CfMMoV*), dog saliva and human sweat. As control no contamination was carried out or no decontamination strategy was carried out. After DNA- and RNA-extraction the recovery rate of contaminants and tick-borne bacterial communities was determined by qPCR.
Our qPCR results showed that 5% NaOCl is the best decontamination strategy followed by DNA away, RSDL and 70% ethanol. Correspondence analyses confirmed our qPCR results. Moreover, bacterial community composition of ticks decontaminated by 5% NaOCl clustered with negative controls in correspondence analysis indicating that removal of tick contaminants was superior. In contrast, decontamination by 70% ethanol were less efficient, which is in line with qPCR results. Further multivariate statistics of amplicon sequencing data will help to evaluate efficacy of reported decontamination strategies.

T&O-15
Detection of questing *Ixodes frontalis* larvae in a forest close to Berlin (Germany) in November 2018

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The dominating hard tick species in central Europe is *Ixodes ricinus*, the castor bean tick, a principal vector of various pathogens. Recent research identified also the newly described *Ixodes inopinatus* as an exophilic tick that is widespread and quite common in Germany.
There has been an ongoing project with monthly flagging excursions (supported by Pfizer Deutschland GmbH), which includes a location close to Berlin. Whereas all the collected tick nymphs and adults have been counted and morphologically determined down to the species level, the overall numbers of flagged *Ixodes* larvae were only estimated, without closer determination.
It was unexpected that, in addition to 2 *I. ricinus* nymphs and 3 *Dermacentor reticulatus* adults, we collected 10 *Ixodes* larvae on the 28th of November 2018 at approximately 0°C. All those larvae were morphologically determined as *Ixodes frontalis*, a species known to be associated with birds but very rarely found in Germany.
This remarkable example emphasizes how important it is to determine field-collected ticks down to the species level and not simply assume that all flagged *Ixodes* larvae in central Europe are *I. ricinus*. With that negligent approach, many *I. inopinatus* and perhaps also members of some other *Ixodes* species might erroneously be scored as *I. ricinus* in field studies what may easily lead to wrong conclusions.

T&O-16
Ticks in the close surroundings of football grounds in Germany, a pilot study

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Ixodes ricinus is the most abundant tick species and an important vector of pathogens in central Europe. In addition, there are further ixodid tick species that occur more or less frequently in that region and may also be of eco-epidemiological relevance, for example *Ixodes inopinatus* and *Dermacentor* ticks. It is well known that tick populations can establish not only in natural but also in suburban green areas (parks, gardens). The aim of the present study was to find out whether ticks are often present nearby football grounds in Germany and pose a potential risk to the players and visitors.
Thirty-two football grounds were selected, most of them situated adjacent to a green area (forest, park). All 16 German states were considered. In each case, the consent of the owner was obtained in advance. Ticks were collected by flagging 100 m2 in the close surroundings (10 drags à 10 m2) plus 2 drags à 10 m2 on each football ground. Only collected tick nymphs and adults were counted and determined, not the larvae.
Altogether 807 nymphal and adult ticks were collected at 29 football grounds, 711 *I. ricinus*, 63 *I. inopinatus*, 2 *I. frontalis*, and 3 *D. reticulatus*. *Ixodes inopinatus* was found in 13 out of 16 German states. Twenty-eight *Ixodes* ticks could not be determined morphologically with certainty, yet, but 3 of these might be *I. festai*. In 3 cases, there were some ticks found even on a football ground.
It can be concluded that ticks occur quite frequently and sometimes in high abundance at football grounds situated close or adjacent to a forest or a park. Players, staff, and visitors should be aware of that risk and take precautionary measures.

T&O-19
Structural and functional characterization of Iristatin, a novel immunosuppressive *Ixodes ricinus* tick salivary cystatin

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To successfully finish their blood meal, ticks secrete a variety of pharmacoactive molecules into their saliva including cysteine protease inhibitors from the cystatin family.
However, the full complement of cystatins in ticks has yet to be described, not least because not all tick genomes are sequenced. Here we present a novel immunomodulatory cystatin, Iristatin, which

is upregulated in the salivary glands of the hard tick *Ixodes ricinus* during feeding. We present the crystal structure of Iristatin at a resolution of 1.76Å. Purified recombinant Iristatin inhibited the proteolytic activity of cathepsins L and C and diminished IL-2, IL-4, IL-9, and IFN-γ production by different T cell populations, IL-6 and IL-9 production by mast cells, and nitric oxide production by macrophages. Furthermore, Iristatin inhibited OVA antigen-induced CD4+ T cell proliferation and leukocyte recruitment *in vivo* and *in vitro*. Our results indicate that Iristatin affects wide range of anti-tick immune responses in the vertebrate host and may be exploitable as an immunotherapeutic.

T&O-20
Epigenetic modifications of the *Ixodes ricinus* genome

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Introduction: In spite of the global medical and veterinary importance of *Ixodes ricinus* relatively little is known about its genome organization and DNA epigenetic modification.
Objective: Analysis of the DNA methylation status and determination of the DNA methylation enzymes transcripts abundances in different life stages of *Ixodes ricinus*.
Materials & methods: *I. ricinus* gDNA and mRNA were obtained from eggs, larvae, partially fed nymphs, unfed, and partially fed females. Methylation-sensitive restriction enzymes MspI, HpaII, DpnI, and DpnII were used to cleave the gDNA. Dot Blot assay with antibodies against N6-methyladenosine (6mA) and 5-methylcytosine (5mC) were used to detect the methylated gDNA. The transcriptome of *I. ricinus* was assembled using Trinity assembler and the relative abundance of transcripts was calculated using RSEM.
Results: The cleavage of gDNA by methylation-sensitive enzymes suggested the presence of cytosine and adenine methylation. Dot blot assay confirmed these data, and both 6mA and 5mC modifications were found in DNA samples isolated from the *I. ricinus* ticks at different life stages. In the transcriptome of *I. ricinus,* 14 transcripts of putative *I. ricinus* DNA methyltransferases were identified. Among them, we identified hypothetical *I. ricinus* DNMT1, DNMT3, and DAMT enzymes, and found that their expression levels varied in different tick life-stages.
Conclusions: DNA methylation was confirmed in *I. ricinus* ticks and the sequences of the respective DNA methyltransferases were identified in *I. ricinus* transcriptome.
This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic INTER-ACTION project LTARF 18021 and the Grant Agency of the Czech Republic projects 15-03044S and 18-27204S. Access to instruments and other facilities was supported by the Czech research infrastructure for systems biology C4SYS (project no LM2015055).

T&O-21

Project OSWALD: First data on tick activity in different land cover classes in Germany

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Introduction: Ticks, especially hard ticks of the genus *Ixodes*, are the most important vectors of human pathogens in Germany. They are capable of transmitting a variety of different pathogens of which TBEV (Tick-borne encephalitis virus) is the most important viral one. Therefore, it is of great importance to get detailed knowledge about the spatial and temporal activity of those vector ticks.
Objectives: The aim of this study is to gather information on the spatial and temporal activity of hard ticks in Germany. This data is provided within the project "OSWALD" to a group modeling tick activity in Germany and creating forecasts. We here present the results of the first year"s survey.
Material & methods: Using the CORINE land cover classification system, 29 regions with a total of 86 sampling sites were chosen all over Germany. The classification of the sampling sites was done using 5 distinct categories, which are agriculture, broadleaf forest, coniferous forest, mixed forest and urban area. Each site was chosen with a size of at least 2 ha to prevent influence of flagging on the tick density.
Ticks were flagged once per month from February till November in an area of 100 m^2. Identification of species and developmental stage was carried out in laboratories.
Results: A total of 13719 *Ixodes* spp. ticks were flagged. Of these, 12293 ticks were nymphs and 1393 adults. The highest tick density was found in broadleaf forests, whereas the lowest number of ticks was collected in urban areas. Main tick activity was recorded during the months of April to June. Additionally, there was a decrease of tick activity going from North to South.
Conclusion: Even though this the first study of such a large scale, data for comparison from additional years is necessary for comparison, as the summer of 2018 was very long and especially dry.

T&O-22
A comparison between *Ixodes ricinus* nymphs fed *in vitro* and on calves

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Ixodes ricinus is a common tick species in Europe and a primary vector for tick-borne diseases such as Lyme borreliosis and tick-borne encephalitis. Artificial tick feeding systems (ATFS) are promising tools for studying the vector biology of this, and other, tick species and an alternative to the use of experimental animals.
In this study, we fed *I. ricinus* nymphs *in vitro* using a modified ATFS and compared the results to that obtained by experimental infestation on cattle. For this purpose, laboratory-reared nymphs, 3 to 12 months after molting, were fed using the ATFS. Glass tick feeding units with a diameter of 20 mm were used, which were closed on one side with a membrane based on a matrix of goldbeater"s skin coated with a silicone mixture, with a thickness of 50-70 μm. Approx. 20 nymphs were placed in each feeding unit, which were hung in the wells of a standard 12-well plate. One mL of sterile, heparinized bovine blood supplemented with gentamycin and adenosine triphosphate was added to each well and changed twice daily. In total, 1990 nymphs were fed in the ATFS. The mean maximum attachment rate was 51 $\pm$ 12%, whereas the mean engorgement rate was 20 $\pm$ 10%. Nymphs of the same cohorts were fed on calves as a control group. The engorgement rate of these nymphs was 53%. While *in vitro* fed nymphs had a mean weight of 3.07 $\pm$ 0.47 mg, which was lower than the mean weight for nymphs fed on calves (3.41 $\pm$ 0.12 mg). The results showed that the artificial feeding of *I. ricinus* nymphs using the modified ATFS requires further optimization, as engorgement rates were relatively low and showed a high variation compared to nymphs fed on calves.

T&O-23
Ticks (Acari: Ixodidae) infesting Cattle in Selected Districts of Uganda, 2017

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Ticks are the most important arthropod vectors and transmit a wide range of pathogens. Tick-borne diseases are a major threat to both cattle and human health with enormous associated economic burden. Currently, there is limited information on tick diversity in Uganda.
We investigated tick species that infest cattle in five districts in Uganda.

In total, 100 cattle were randomly selected from each of the districts of Kasese, Hoima, Gulu, Soroti and Moroto. Adult ticks were handpicked from the animal and transported to Uganda Virus Research Institute, Entebbe, Uganda, for morphological identification to species level. A proportion of ticks from each of the identified species was sent to the Swedish University of Agricultural Sciences, Uppsala, Sweden, and the Bundeswehr Institute of Microbiology, Munich, Germany, for morphological and genetical validation.
Totally, 500 cattle were included in the study. A total of 4,317 ticks were collected, and preliminary results from the morphological identification of 3,842 (89%) ticks, three genera and 13 species were identified as: 7 *Rhipicephalus* species (*Rhipicephalus. appendiculatus*, *Rhipicephalus evertsi evertsi*, *Rhipicephalus turanicus*, *Rhipicephalus pulchellus*, *Rhipicephalus decoloratus*, *Rhipicephalus simus*, *Rhipicephalus microplus*); 4 *Amblyomma* species (*Amblyomma variegatum*, *Amblyomma lepidum*, *Amblyomma cohaerens* and *Amblyomma gemma*); and 2 *Hyalomma* species (*Hyalomma rufipes* and *Hyalomma truncatum)*. *R. appendiculatus* was the most common species (2,067/3,842; 53.8%), followed by *A. lepidum* (700/3,842; 18.5%). Whereas species diversity was highest in Moroto district, regional predominance by specific ticks was observed.
Cattle keeping remain a major socio-economic activity and source of food for many Ugandans. This study demonstrated that cattle are infested by multiple tick species, and are potentially a significant source of many tick-borne pathogens.

T&O-26

The effectiveness of the environmental *Metarhizium anisopliae* strain against the local *Ixodes ricinus* population

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Introduction: Entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* are frequently used for the control of agricultural and forest pests. However, the data on their effectiveness against ticks are scarce and were focused mainly on *Ixodes scapularis, Rhipicephalus sanguineus*, *R. appendiculatus, R. microplus*, *R. annulatus*, *Hyalomma excavatum, Dermacentor variabilis* and *Amblyomma variegatum*.
Objectives: The aim of the study was to assess the bioactivity of environmental *Metharizium anisopliae* strain against local *Ixodes ricinus* population.
Materials & methods: The entomopathogenic fungi were obtained from soil collected from the recreational area of the Osobowicki Forest (Wrocław, SW Poland) using the insect bait method (Zimmerman 1986). The species identification was carried out based on morphology and molecular method (sequence analysis of ITS4 and ITS5). In addition, API ZYM (Biomerieux) and Chitinase Assay Kit (Sigma) were used to identify enzymes produced by this fungal strain. The strain with the highest chitinase production, *M. anisopliae* strain 47(3), was selected to bioassay, ie. to estimate its effectiveness against females and males of *I. ricinus* ticks collected by the flagging method in Wroclaw agglomeration.

For the bioassay four dilutions of conidia were used and 100 adult ticks (separately 10 females and 10 males for each dilution and for the control). Tick mortality observations were made daily over the course of 3 weeks.
Results: At the highest conidia concentration of *M. anisopliae* 47(3) (10 ^ 8 conidia/mL), the mortality rates of *I. ricinus* ticks reached 80% for females and 70% for males. LC50 value for females was 9x10 ^ 5 cfu/ml and 1,1x10 ^ 6 cfu/ml for males.
Conclusion: Environmental fungal strains of *M. anisopliae* obtained from soil appeared to have some potential as a biological agent for control of *I. ricinus* local population.
The study was supported by University of Wroclaw (grant number 0420/2308/17)

T&O-27
Geographical distribution and climate adaptation of the Eurasian hard tick *Haemaphysalis concinna*

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Up-to-date maps depicting the geographical distribution and climate adaptation of *Haemaphysalis concinna* (Koch, 1844) were missing until recently. Here the entire so far known distribution of *H. concinna*, a proven vector of the TBE virus, is presented ranging from the Spanish Atlantic coast to Kamchatka, far-eastern Russia, mainly within 28-64° N latitude. The herein used dataset consists of 656 georeferenced locations in Eurasia. Special emphasis is on Central Europe, where *H. concinna* is the third most abundant hard tick species flagged from vegetation. To investigate its climate adaptation, the georeferenced locations were superimposed on a high-resolution map of the Köppen-Geiger climate classification. The frequency distribution of the tick"s occurrence in the different climates shows three peaks related to the following types: (i) warm temperate with precipitation all year round, (ii) boreal with precipitation all year round, and (iii) boreal, winter dry, climates. Almost 87 % of all *H. concinna* locations are related to these climates. The remaining locations are characterized by cold steppes and deserts, Mediterranean climates, or warm temperate climates with dry winters. As examples, high-resolution maps for Vienna, Novosibirsk and Irkutsk are presented together with their climate diagrams. They show similar temperature and precipitation conditions during May to September, the main seasonal activity period of the parasitic life stages of *H. concinna*.

T&O-28

Preliminary results for diurnal questing activity of *Ixodes ricinus* in Leipzig, Germany

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Introduction: It is broadly assumed that temperature and humidity, and thereby season, have the most significant influence on the questing activity of *Ixodes ricinus*. However, there is almost no research published on how its questing activity fluctuates during the day and which external factors influence this diurnal rhythm.
Objective: The objective of our study was to find out how the questing activity of *I. ricinus* in its natural habitat varies in relation to time of day, light conditions, temperature, and season. Because many of the ticks' hosts are mainly nocturnal or crepuscular, we were particularly interested in tick activity during dawn and night.
Material & methods: From June to November 2018, ticks were flagged every four hours, six times a day, once per month, over a 100m distance at three different sites using a $1m^2$ cotton flag. During each sampling, temperature, weather and light conditions were recorded. Collected ticks were identified to species level under a stereo microscope using taxonomic keys and frozen at -80°C.
Results: In total we flagged 419 *I. ricinus* (357 nymphs, 40 females, 22 males). The monthly variation in questing activity followed the typical bimodal pattern in all locations. In one spot, significantly more ticks quested during daytime compared to darkness. In the other spots, total tick numbers were small and no significant differences could be found. During the short study period, tick questing activity between the six points in time did not differ significantly.
Conclusion: Though the larger amount of questing activity of *I. ricinus* in our study occured during daylight, a substantial portion happened during darkness. There are hints that the questing activity at night might depend on the characteristics of the flagging spot. Therefore, we will continue the study in 2019 in order to obtain larger numbers of ticks for the statistical analysis.

T&O-30

Nonspecific prophylaxis of natural focal diseases caused by ticks *Ixodes spp.* In Russia

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Introduction: There is wide area with suitable habitat for ticks *Ixodes spp.* in Russia. Around 2-4 thousand cases of tick-borne encephalitis, 6-10 thousand Lyme borreliosis and other mixed infections were registered annually for the past 10 years.
Aim of the study: Efficacy assessment of the modern activities aimed at reducing the number of Ixodid tick bites in Russia.
Methods: The use of multi-year acarological studies in natural foci of tick-borne infections in the Irkutsk region and state organizations" statistic data.

Results: In Russia acaricidal treatments of natural biotopes are carried out annually in 80-170 thousand ha area, with the use of cypermethrin-, alpha-cypermethrin-, cyfluthrin-, deltamethrin- and fenthion-based insecticides. The effectiveness of these measures is high (over 95%), but the acaricidal effect lasts 1-1.5 months max. The *Ixodes spp.* ticks resistance to applied acaricides is negative. The treatments are carried out after the acarological survey in the urban green spaces and especially in the suburbs: around nursing homes, health centers, and children's health facilities. In the rest of the territory it is recommended to use acaricidal and repellent products as a spray on outdoor clothes. Our studies show the highest protective properties of alpha-cypermethrin-based acaricide-repellent products. Special clothing designed to protect against arthropods has the most complete protective capability. This clothing became widely used especially in the tourism fields, power industry, oil and gas production - the fields with expected long-term being in forest biotopes.
Conclusion: Acaricidal treatments are considered to be effective in places of the high risk of human-tick contact. Educating people about the correct behavior in tick-infested areas and correct use of special protective products are also considered to be effective. It is necessary to continue improving the efficiency of resources and methods of anti-tick protection for people.

T&O-31

A proposed biological mechanism of increased aggressiveness of ixodid ticks under high ambient temperatures

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It is documented that the frequency of human attacks by all parasitic stages of *Rhipicephalus sanguineus* rises with increasing temperatures, which is also supported by epidemiological data on human cases of tick-transmitted rickettsioses. We observed a similar relationship in adult *Ixodes persulcatus* during regular tick collection in the Sayan Mountains: the ratio of the number of ticks detected on collectors to their number on flags was greater at higher daytime temperatures.
It is important to keep in mind that air temperature negatively correlates with air humidity. We were able to compare the ratios of ticks on collectors to ticks on flags under the same temperatures but at contrasting humidity levels. When humidity was lower, the proportion of ticks found on collectors' clothes (especially on the upper parts) was significantly increased. Since elevated temperatures are usually associated with lower air humidity which, in turn, increases water loss by ticks, such conditions are expected to favor tick behaviors aimed at compensating dehydration. Typically, when air humidity is low ticks move down to the litter level to restore their water content. We propose that when ticks sense the proximity of a host, they activate an alternative behavioral approach to restoring hydration by expediting their attachment to the host. Since the water content of the blood gorged by ticks is about 80%, it is reasonable to hypothesize that the elevated aggressiveness of ticks at higher temperatures is primarily driven by reduced air humidity rather than being a direct effect of the temperature itself.
This hypothesis readily explains the aggressiveness of *R. sanguineus* towards humans.

Opportunities for rapid water replenishment in the mostly urban habitats of this species are limited, so even though the affinity of these ticks for humans is usually low, at high ambient temperatures they have little choice but to aggressively attack any suitable host to stave off desiccation.

T&O-32
Seasonal activity of hard ticks in Vienna

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Hard ticks (Ixodidae) play an important role for public health as they are vectors for pathogens, causing diseases as tick-borne encephalitis or Lyme borreliosis. In Austria the species *Ixodes ricinus* is the main disease vector. Also the lately described new species *I. inopinatus* are possible vectors. For reliable risk assessments of tick bites in humans and animals, ticks should be monitored in terms of their ecology, occurrence, and density. To determine the seasonal activity of ticks in Vienna and surroundings, a monitoring in three popular recreation areas (Prater, Kahlenberg, Klosterneuburg) were established. Using the flag method, ticks are collected on the monthly base since May 2017. Therefore, a white 1x1 m^2 flag is striped over the vegetation to flag an area of 100 m^2. Afterwards the ticks are identified morphologically using common identification keys. Between May 2017 and November 2018, a total of 485 nymphal and 87 adult hard ticks per 100 m^2 were collected. In a natural garden with game paths in Klosterneuburg, the highest tick densities (58 %) were observed each month, followed by the site Prater, a large public park with a riparian zone (26 %), and the site Kahlenberg, a broadleaf forest with a dense layer of trees and a small amount of herb layer (16 %). The main focus of the morphological determination lied on the distinction between *I. ricinus* and *I. inopinatus*. Pooled over all three sites, the proportion of *I. ricinus* and *I. inopinatus* was 92 % to 6 %, only a small proportion of 2 % remains unclear as it could not be assigned morphologically to a species. During the hot summer month also *Haemaphysalis concinna* nymphs and adults were collected at all the three sites.

T&O-33
The problem of *Ixodes* ticks in parks of Moscow city: ways of solution

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Introduction: The problem of blood-sucking ticks as vectors for infectious human diseases is an important issue for many cities around the world including Moscow City with over 70 parks in its areas.
Aim of the study: Define the problem of ixodes ticks in Moscow parks and form main ways of nonspecific tick-borne infection prophylaxis in city parks.
Methods: Data analysis of reported tick-borne diseases and cases of tick bites across Moscow area was performed. Acarological research of the natural biotopes was made using GPS markers in tick populated areas. The parks investigated in 2016-2018 are Sokolniki, Serebryany Bor, Zamoskvoretsky,

Bitsa, Losiny Ostrov National Park. Pathogens in ticks were identified by PCR analysis. Citizens" knowledge on ticks and related diseases was evaluated on the surveys in social media and in the parks.
Results: 5761 reports of tick bites in Moscow parks and 203 cases of Lyme borreliosis with contamination in the parks were registered for the past 5 years. The cases of tick-borne encephalitis were imported. *Ixodes ricinus* ticks type was dominating, *Dermacentor reticulatus* was encountered less rarely. The most tick heavy parks are Serebryany Bor and Losiny Ostrov with two main activity periods: spring-summer and autumn. Next pathogens were found in ticks: *Borrelia spp., Rickettsia helvetica, Anaplasma phagocytophilum*, Kemerovo virus. On 3430 Moscow citizens" surveys 58% were aware of tick-borne diseases and preventive measures, though only 68% of them used individual anti-tick protective means.
Conclusion: For the large number of park visitors and urban green areas extension in Moscow, ticks population status should be monitored constantly. To lower the risk of human-tick contact in parks the work for educating people about ticks, tick-borne diseases and individual anti-tick protective resources should be conducted. Issues of ecological rearrangement of park areas for visitors' biosafety should be addressed by the city management.

T&O-35
Comparison of *Ixodes ricinus* populations in adjacent habitats on a pasture-based dairy farm

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Introduction: The distribution of *Ixodes ricinus* ticks in the environment tends to be highly overdispersed with greatly divergent densities in adjacent habitats. In order to (1) investigate the mechanisms that underlie this overdispersion and to (2) assess the animal and public health risks associated with the different habitats, we compared tick populations in adjacent habitats with regard to tick abundance, the most recent engorgement host and the prevalence of tick-borne pathogens (TBPs).
Methods: Five adjacent sites on a dairy farm in Co Kerry were sampled for the presence of ticks using standard blanket dragging methods. From each site, 15 nymphs were analysed for C and N stable isotope compositions and 40 were screened for the presence of TBDs using Taqman qPCR analysis.
Results: Both nymphs and adult ticks were most abundant in hedges bordering the pasture, somewhat less common at the edge of the path and in the woodland and absent from the centre of the pasture. Isotope analysis indicated that the nymphs that quested in the woodland had fed

on the broadest range of hosts, while qPCR analysis suggested that nymphs collected from the hedge that separated the woodland from the pasture had the highest prevalence of TBPs (*Anaplasma phagocytophilum*, *Babesia* (cattle or deer spp) and *Borrelia* spp).
Discussion & Conclusion: While there was some overlap in the feeding guilds of hosts parasitized by nymphs in the different habitats, nymphs in the hedge bordering the woodland seemed to be chiefly feeding on one guild (possibly ruminants) while nymphs in the woodland fed on a broader range of hosts (probably rodents and birds in addition to ruminants). The results also suggest that ticks that detach from livestock or deer on pasture move into the hedge, boosting the tick numbers and increasing the prevalence of livestock TBPs in ticks in this site.

T&O-37

Screening for novel *I. ricinus* vaccine candidates by probing a novel *I. ricinus* salivary gland Yeast Surface Display with sera from forestry workers

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Since 1939 evidence has accumulated indicating that repeated tick infestations can lead to 'tick immunity' and protect against *Borrelia burgdorferi* infection in laboratory animals. In humans, repeated tick-bites, and itch after a tick-bite, are associated with a reduced likelihood of contracting Lyme *borreliosis*. We here aimed to identify tick salivary gland proteins that could be involved in human anti-tick humoral immune responses. A cDNA library constructed of combined salivary gland RNA from nymphal *I. ricinus* ticks feeding for 24, 48 and 72 hours was cloned into the pYD1 vector and transformed into *S. cerevisiae* EBY-100 cells. IgG of 22 Dutch Forestry workers (FWs) that reported more than 20 tick bites per year was used to enrich the YSD with three subsequent MACS sorting rounds. Although only 2% of the initial library bound to the FWs IgG, the percentage increased to 17% after MACS sorting. In contrast, pooled IgG of controls (clerk personnel) did not show an increase in reactivity. Next, the enriched yeast cells were labeled with FWs IgG and single cells were sorted by flow cytometry. Subsequently, plasmids were isolated, sequenced and the amino acid sequences were BLASTed against the UNIPROT database. With this approach 12 proteins were identified: 2 housekeeping, 5 glycine rich, 2 RNA- binding, 1 cuticle and 2 putative salivary secreted proteins. FACS analysis of representative clones confirmed that these proteins were recognized by FWs, but not by controls. Thus, the identified tick salivary gland proteins are immunogenic in humans, might be involved in 'human tick immunity' and could therefore be anti-tick vaccine candidates. Three vaccine candidates have been tested in rabbit and cow vaccination studies, but did not show a significant effect on tick feeding parameters for nymphal and adult *I. ricinus*.

However, as vaccination might be able to affect *Borrelia* transmission in other ways than through feeding success, their effect on *Borrelia* transmission is currently being studied.
This project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No. 602272.

T&O-39
Identification of novel *Ixodes* vaccine candidates using Yeast Surface Display technology

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Ixodes ticks transmit bacterial, protozoal and viral pathogens, causing disease and becoming an increasing health concern in Europe. It is known that repeated tick infestations can lead to 'tick immunity', which leads to reduced tick feeding and partially protects against *Borrelia burgdorferi* infection in laboratory animals. *Ixodes ricinus* and *Ixodes scapularis* are closely related and bioinformatic analysis shows that approximately 54% of *I. ricinus* transcripts have an identity to *I. scapularis* transcripts higher than 80%. In the current study, a cDNA library constructed of combined salivary gland RNA from nymphal *I. ricinus* ticks feeding for 24, 48 and 72 hours has been cloned into the pYD1 vector and transformed into *S. cerevisiae* EBY-100 cells. Rabbits were repeatedly exposed to *I. scapularis* nymphs feeding to repletion, or *I. ricinus* nymphs feeding for 24 hours, and sera were collected 2 weeks after the last infestation. Purified IgG was used for two screening strategies: 1) MACS enrichment and subsequent single cell FACS sorting using tick immune *I. scapularis* rabbit IgG. 2) MACS enrichment with IgG raised against 24h feeding *I. ricinus* nymphs followed by single sorting by flow cytometry using tick immune *I. scapularis* rabbit IgG. Plasmids of isolated single yeast cells were isolated, sequenced and the amino acid sequences were BLASTed against the UNIPROT database. With this approach 13 proteins have been identified that have highly conserved *Ixodes* epitopes and are very likely to be involved in 'tick immunity'. These proteins could be excellent vaccination candidates for a vaccine targeting both *Ixodes ricinus* and *Ixodes scapularis* and might prevent their associated diseases. Six vaccine candidates have been tested in rabbit vaccination studies, but did not show a significant effect on tick feeding parameters for nymphal and adult *I. ricinus*. However, as vaccination might be able to affect *Borrelia* transmission in other ways than through feeding success, their effect on *Borrelia* transmission is currently being studied.
This project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No. 602272.

POSTER ABSTRACTS
Tick-borne Encephalitis Virus | TBEV-1 – TBEV-17

TBEV-1
CRYPtick: Understanding the recent emergence of TBEV in the Netherlands

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Tick-borne pathogens have a significant impact on human and animal health. The most important arboviral disease in Eurasia is caused by tick-borne encephalitis virus (TBEV), which is endemic in large parts of Eurasia and has recently emerged in The Netherlands. The ecological and molecular factors underlying this emergence remain poorly understood. Important factors that need to be addressed are: 1) the vector competence of ticks, 2) the existence of a possible cryptic TBEV transmission cycle, and 3) the influence of TBEV on tick behaviour. In this study, we will examine each of these three issues. We will study the vector competence of Dutch *Ixodes ricinus* ticks for TBEV-Neudoerfl, TBEV-Salland and Louping Ill virus. Furthermore, the potential effects of TBEV co-infection with other tick-borne pathogens (e.g., *Borrelia burgdorferi*) on the vector competence will be quantified. With our Biosafety Level 3 facilities, we will be able to study TBEV transmission and virus-tick interactions *in vivo*. With the use of an artificial membrane for blood-feeding ticks, we will infect nymphs with TBEV via a natural way. Besides vector competence and co-infection interactions, we will also experimentally test whether and how TBEV manipulates tick behaviour, affecting virus transmission. The main route of TBEV transmission is assumed to be via co-feeding on rodents between infected nymphs and uninfected larval ticks. Besides co-feeding, other TBEV-transmission cycles have been hypothesized, and we will therefore study the potential role of pheasants, hares, hedgehogs (important hosts to ticks) and the hedgehog tick *I. hexagonus* in TBEV transmission. Studying both ecological and molecular factors influencing TBEV transmission is important for our understanding of TBEV emergence in the field.

TBEV-2
The effect of tick saliva on the replication of tick-borne encephalitis virus in different murine macrophage cell lines

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Tick-borne encephalitis virus (TBEV), a causative agent of a serious neurological disease, is transmitted to human generally through the bite of an infected tick. Virus is transported via tick saliva that has immunomodulatory effects on host defence mechanisms and creates a unique environment

for pathogen transmission. Macrophages play a key role against invading pathogens but are also susceptible to infection and can serve as an important replication site for TBEV.
Objectives: Our aim was to characterize the interaction of TBEV and mouse macrophages and how is this interaction affected by tick saliva from *I ricinus*. The role of type I interferon (IFN-β) and nitric oxide (NO) in viral replication was elucidated.
Materials & methods: Three mouse macrophage cell lines (PMJ2-R, IC-21 and P388/D1) were infected with TBEV strain Hypr. Viral replication was determined by plaque assay and the proportion of infected cells was assessed by immunofluorescence staining. IFN-β production was measured by ELISA and the amount of NO was determined using the Griess method.
Results: All tested cell lines were permissive to TBEV infection with evidently different susceptibility among them. In the presence of tick saliva, we observed at early times after infection (24 hpi) significant decrease in viral replication and in the percentage of infected cells. All cell lines produced IFN-β in response to virus infection, but the production was delayed till 48 hpi. In addition, the IFN-β level was significantly increased in the presence of saliva. No nitric oxide production was detected in TBEV-infected cells.
Conclusion: We present here that three various macrophage cell lines differ in their permissiveness to TBEV infection. We show that tick saliva has inhibitory effect on the replication of TBEV. Because saliva-induced decline in TBEV replication preceded the production of IFN-β, we suggest that negative effect of tick saliva on TBEV replication in macrophages is no mediated by IFN-β.

TBEV-3
Comparative analysis of the tick-borne encephalitis virus load in ticks

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Introduction: Tick-borne encephalitis is one of the most important human infectious diseases of the central nervous system in Europe and Asia. The infectious agent, the tick-borne encephalitis virus (TBEV) is a member of the flaviviruses and is transmitted to humans via tick bites or the consumption of raw dairy products. So far, there are few quantitative data available how many virus is contained in ticks and whether the amount of transmitted virus might influence the severity of human disease.
Methods: In order to obtain data about the virus load of ticks, in collected RT-qPCR positive ticks, the TBEV load was determined for every individual sample using plaque assays. The CT-values of the semi-quantitative RT-PCR were compared with the individual viral loads of each sample. We further compared the TBEV loads from different sampling sites with each other. The viral loads of samples from different tick stages were analysed as well. Furthermore the viral loads were recorded over time to see whether the weather conditions of a year might influence the amount of TBE virus found in ticks.
Results: So far we can see no clear correlation between the CT-values of the PCR and the virus titres of the plaque assays. There is a clear correlation between the virus load and the sampling location though. While in the sampling site of Heselbach the samples had an average viral load of 7.58 E+5 plaque forming units, in the sampling location of Mühlau, the average viral load was 5,13 E+03. Adult ticks have a higher viral load than nymphs. Further the viral load in female ticks is higher than in males.

Discussion: Our findings indicate that the sampling location and therefore the virus strain may play the most important role concerning the viral load of the ticks. The differences in stage and sex warrant further studies and might be of importance in the natural transmission cycle of the TBEV.

TBEV-4
Biotyping of TBEV-infected IRE/CTVM19 cells

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Introduction: Ticks, as vectors for a variety of pathogens such as tick-borne encephalitis virus (TBEV), have developed defense mechanisms and pathways against the transmitted infections, allowing them to control the virus at a level which does not hinder their fitness and development. Currently, there have been published only few studies dedicated to the investigation of interactions between the tick and TBEV on a molecular level.
Objectives: Investigation of differences in MS profiles of IRE/CTVM19 cells during TBEV infection.
Materials & methods: Biotyping was performed on an Autoflex Speed MALDI-TOF/TOF (Bruker Daltonik). Protein digests were analysed using Synapt G2-SiHigh Definition mass spectrometer.
Results: Principal component analysis (PCA) of MS profiles revealed a distinct cluster for 2 days post infection (dpi) cells, whereas 5 dpi and 10 dpi cells were grouped together. PCA showed that 25 peaks have an influence on grouping of MS spectra, of which 12 peaks were common for all cell states throughout time, and 13 peaks were significant in terms of infection duration. Eight characteristic signals in MS profiles of IRE/CTVM19 cells were assigned with proteins identified in the corresponding protein extracts by nanoLC-ESI-MS/MS.
Conclusion: Possible protein markers of IRE/CTVM19 cells were identified, as well as markers of late TBEV-infection periods in the IRE/CTVM19 cells.
This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic INTER-ACTION project (LTARF 18021); GAČR (18-27204S), European Regional Development Fund Project “Mechanisms and dynamics of macromolecular complexes: from single molecules to cells” (CZ.02.1.01/0.0/0.0/15_003/0000441), and the Ministry of Science and Higher Education of the Russian Federation (agreement #14.616.21.0094, unique identifier RFMEFI61618X0094). Access to instruments and other facilities was supported by the Czech research infrastructure for systems biology C4SYS (LM2015055).

TBEV-5

Isolation of Tick-Borne Encephalitis virus from *Dermacentor reticulatus* and *Ixodes ricinus* in an endemic area in Germany

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Introduction: Tick-borne encephalitis (TBE) virus is transmitted to humans and animals through tick bites and is thought to circulate in very strictly defined natural environments called natural foci. The most common tick serving as a vector for TBE virus in Central Europe is *Ixodes ricinus*, rarely it is found in other tick species and, so far, in *Dermacentor reticulatus* ticks it has only been reported in Poland.
Objectives: To describe a TBE focus and the TBE virus in *D. reticulatus* for a period of three seasons.
Material & methods: Between autumn 2016 and spring 2018 ticks were collected by the flagging method in a new TBE focus in the district of northern Saxony, Germany. Ticks were morphologically identified and tested in pools for the presence of TBE virus using a real-time RT-PCR. TBE virus from positive pools was isolated in A549 cells, and the E gene sequences were determined after conventional RT-PCR, followed by a phylogenetic comparison.
Results: TBE virus was detected in eleven pools, nine times in flagged adults *D. reticulatus* (n=1,534; MIR: 0.59%, CI: 0.29-11.3), and twice in *I. ricinus* nymphs (n=349; MIR: 0.57%, CI: 0.02-2.2). All other ticks, *I. ricinus* males (n=33), females (n=30), and larvae (n=58), as well as five *I. inopinatus* (2 females, 3 males) and 14 *Haemaphysalis concinna* (3 females, 11 nymphs), tested negative for TBE virus. TBE virus was not detected in *I. ricinus* during the summer, when *D. reticulatus* was not active. Sequence comparison of the entire E gene of the isolated virus strains resembled each other with only 3 nucleotide differences. The most closely related viral sequences belong to TBE virus strains from Poland and Neustadt an der Waldnaab, about 200 km east and 200 km south-west of the focus.
Conclusion: TBE virus was found in this region with similar MIRs in *D. reticulatus* and *I. ricinus*, indicating that *D. reticulatus* seems to play an equal role in a virus circulation as *I. ricinus* when both tick species occur sympatrically.

TBEV-6

Temporal phenology and TBE virus prevalence of *Ixodes* ticks in a German TBE focus over 10 years

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Introduction: Tick-borne encephalitis (TBE) is the most important tick-transmitted viral disease in Eurasia. In Germany TBE virus is circulating in natural foci, where it circulates between ticks and rodents. The dynamics of these natural foci is yet unclear. Understanding the cyclic transmission could help to facilitate the forecasting of TBE infection years.

Objectives: Aim of the current study was to analyze the phenology of ticks in a TBE natural focus and the prevalence of TBE virus in ticks (*Ixodes ricinus, Ixodes inopinatus*) over a period of 10 years to detect cycles of occurance of virus in relation to ticks.
Methods: The TBE natural focus of Haselmühl was sampled monthly during the major tick activity (mainly March to October) over a period of 10 years (2009 to 2018). Ticks were pooled according to stage and sex and, from 2017 on, also differentiated in *Ixodes ricinus* and *Ixodes inopinatus*. Tick pools were tested for TBE virus using qRT-PCR (Schwaiger & Cassinotti, 2004) and positive tick pools were confirmed by virus cultivation and sequencing of the E gene of the TBE virus.
Results: Numbers of sampled nymphs varied more than threefold (maximum 150 nymphs on May 2012 to 529 nymphs on May 2018). The number of females and males was rather stable during the whole study period with yearly maxima ranging from 50 to 100 ticks. TBE virus E gene sequencing revealed two different and distantly related TBE virus strains circulating at the same time in the focus. The virus prevalence rates in nymphs was ususally between 0.5 to 1% (except 2015 with 4%). Positive nymphs were detected every year. The adults were not found positive every year. The virus prevalence in adult stages was similar in most "positive" years ranging from 1 to 6%.
Conclusion: The phenology of ticks so far does not follow any repeating cycle. The number of positive ticks does not correlate with the number of ticks. Other factors seem to drive the natural transmisssion cycle of TBE virus.

TBEV-7
BONCAT and Click-reaction-on-membrane as a method for identification of differently expressed proteins during TBEV infection of human neural cells

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Introduction: Changes in protein expression as a response to viral infection can be monitored using many approaches, however, many of them cannot distinguish newly synthesized proteins from preexisting protein pool. We took advantage of BONCAT (bioorthogonal non-canonical amino acid tagging), which is based on incorporation of the unnatural amino acid L-azidohomoalanine (AHA) into newly synthesized proteins instead of L-methionine. For further analysis, AHA-containing proteins are tagged with a biotin tag by the so-called Click reaction. The biotin moiety is subsequently used for visualisation.
The objective of this work was the comparison of 2D electrophoretic protein profile between infected and control samples of human glioblastoma cell line infected with tick-borne encephalitis virus, strain Neudoerfl (TBEV).
Materials & methods: Human glioblastoma cell line was infected with TBEV and newly synthesized proteins were labelled with AHA in a 2 hour window in various intervals after infection.
Proteins were separated using 2D electrophoresis followed by electroblotting. The Click reaction with biotin-alkyne was performed on a PVDF membrane and the tagged proteins were visualized using streptavidin conjugated with alkaline phosphatase. Protein spots from 2D gels were identified using MALDI-TOF/TOF.

Results: Our optimized workflow (performing Click-on-membrane) resulted in improvement of detection of newly synthesized proteins. Differences in protein patterns between control and infected samples were observed. Furthermore, we verified the ability of BONCAT in combination with Click-on-membrane for detection of differentially produced proteins during TBEV infection of human neural cells, some of which were identified by MALDI-TOF/TOF.
Conclusion: In conclusion, we verified the ability of BONCAT in combination with Click reaction on the blotted proteins on membrane for identification of differently produced proteins during TBEV infection of human neural cells.

TBEV-8
Detection of temperature-sensitive tick-borne encephalitis virus strains in natural isolates generated from ticks

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Introduction: Tick-borne encephalitis virus (TBEV) belongs to the family of *flaviviridae* and is the agent causing tick-borne encephalitis. There are three known subtypes, the Siberian, the Far-Eastern and the European. The European Subtype is mainly distributed in Central Europe, but can also be found in the Baltic area and some eastern parts of Europe. There is still discussion on the different pathogenicity of TBEV in nature. Temperature-sensitive TBEV isolates from environment and from the lab were associated with reduced pathogeny in mice.
Methods: During this project we screened 16 different TBEV strains for the ability to replicate at different temperatures in the cell line A549, an adenocarcinoma cell line of human origin. The tested strains included isolates from ticks, mice and the reference strain Neudoerfl.
Results: We were able to identify two TBE virus strains with a decreased capacity to replicate at 40°C by 2 log10. These strains were further cultivated at 40°C to isolate the non-heat sensitive subpopulation of the virus strains. The nucleic acids were isolated and sequenced to identify differences between the temperature sensitive and non-sensitive strains. The obtained sequences were compared to sequences from already known temperature-sensitive strains to further pinpoint to the probable genetic causes of heat-sensitive mutations and possibly also on pathogenicity.
Discussion: These findings contribute to uncovering the diversity of TBEV phenotypes in natural foci and studying the influence of defined mutations for the phenotype and pathogenicity of TBEV.

TBEV-9

Antiviral effect of resveratrol and its derivatives on tick-borne encephalitis virus

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The naturally occurring stilbenes can be found in plants, such as wines, berries, legumes, or pines. Antiviral, antibacterial, anti-oxidative, and anti-inflammatory effects were proven for some stilbenes, including resveratrol (RSV) and its derivatives piceid (PIC) and ε-viniferin (VIN).
Tick-borne encephalitis virus (TBEV) causes more than 10,000 cases of tick-borne encephalitis annually in Eurasia. Despite the existing vaccine against TBEV, this number still rises, due to the low number of vaccinated people. Furthermore, there is no known treatment for the tick-borne encephalitis. Aims of this study were to examine the antiviral effect of RSV, PIC, and VIN on TBEV *in vitro* and *in vivo* in laboratory mice (BALB/c).
Materials & methods: The MTT viability assay was used for the determination of cytotoxic concentrations of the studied stilbenes and for the detection of inhibition of cytopathic effect in two variants of stilbene treatment, one day before the infection (S-->TBEV) or on the same day (S+TBEV). Relative quantification of viral RNA was performed using one-step qRT-PCR and viral titers were calculated using plaque titration in cells treated with the effective concentrations. Mice were infected with TBEV and the stilbenes were injected in both variants. Moreover, the effect of interferon-β in combination with stilbenes was studied.
Results: The cytotoxicity of RSV, PIC, and VIN was detected at concentrations higher than 12.5, 50, and 125 μg/ml, respectively. All chosen stilbenes in variants S+TBEV or S-->TBEV inhibited the cytopathic effect of TBEV and strongly decreased the amount of viral RNA *in vitro*. Pretreatment with VIN reduced the TBEV titer. Stilbenes improved the survival of mice about 2 days on average using low stilbenes dosage. Moreover, pretreatment with interferon-β together with RSV prolonged mice survival by about 8 days.
These results suggest that RSV, PIC, and VIN can be used as the antiviral prophylactic substance against TBEV.

TBEV-10
Tick-borne encephalitis virus in cows and unpasteurized cow milk from Norway

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Introduction: Tick-borne encephalitis virus (TBEV) is mainly transmitted to humans and animals from ticks. However, alimentary TBEV-infections after consumption of unpasteurized dairy products have been reported in Europe. Consumption of unpasteurized dairy products seems to be an increasing trend. In Norway, the incidence of tick-borne encephalitis (TBE) is low, with a total of 169 reported cases between 1997 and 2018. The human cases of TBE are limited to southern Norway, but TBEV RNA in ticks has been detected outside this area. The epidemiology of TBEV in domestic ruminants in Norway has not been fully established, and human alimentary TBE cases have not yet been reported.
Objectives: The objectives of this study were to analyze unpasteurized cow milk from Norway for TBEV RNA and presence of antibodies to TBEV in serum from the same animals.
Materials & methods: Milk and blood samples were collected from a total of 112 cows at five farms in Norway. The milk samples were analyzed by RT real-time PCR and the serum samples were screened by ELISA and verified by neutralization test.
Results: TBEV RNA was found in 5.4% of the unpasteurized milk, from three farms. Neutralizing antibodies to TBEV were found in animals at one farm only, where 88.2% of the cows were positive.
Conclusion: This is the first report of TBEV in unpasteurized cow milk in Norway. TBEV RNA was detected in raw milk collected from areas both with and without reported human TBE-cases. Further studies on milk containing TBEV should be performed to conclude if TBEV found in unpasteurized milk in Norway is infectious to humans.

TBEV-11
Prevalence of Tick-borne encephalitis virus in *Ixodes ricinus* and *Dermacentor reticulatus* ticks in Lithuania

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Tick-borne encephalitis (TBE) is a typical zoonosis disease mainly transmitted to humans by hard ticks Ixodidae family, mainly *Ixodes ricinus* and *Ixodes persulcatus*. It is known that also *Dermacentor reticulatus* is involved in circulation of TBE virus (TBEV). Tens of thousands of people are hospitalized in the world every year, so it has become an international public health problem. One

third of European TBE cases are detected in the Baltic States. Lithuania is one of the countries with the highest number of reported TBE cases in Europe: an average of 450 cases of TBE are reported every year. Distribution of TBE cases in Lithuania varies between different regions and the highest annual incidences of disease are registered in northern and central part of the country. The aim of this study was to investigate the current prevalence of TBEV in ticks and genetically characterize the strains of virus distributed in Lithuania. From March-September 2017 and April-October 2018 ticks were collected from different Lithuanian counties and analyzed for the presence of TBEV. A total 5033 ticks (4001 *I. ricinus* and 1032 *D. reticulatus*) were collected and grouped in 544 pools. For the detection of TBEV a quantitative real-time Revers transcription-PCR (RT-PCR) was performed. Samples positives by real-time PCR were used for one step RT-PCR and for nested PCR for future sequencing of the partial E protein and NS3 genes. Twenty-three (4.2%) pools were found positive for TBEV, with an overall estimated minimum infection rate (MIR) of 0.45%. TBEV was detected in both tick species with MIR 0.58% in *D. reticulatus* and 0.42% in *I. ricinus.* TBEV-infected ticks were found in five counties (Vilnius, Kaunas, Marijampolė, Alytus, Šiauliai) with MIR ranged from 0.11% to 1.84%. The phylogenetic analysis has shown that detected strains belong to the European subtype but have some specific genetic variants.

TBEV-12

Tick-borne encephalitis virus as one of the components of transmission-blocking anti-tick vaccines.

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Tick-borne encephalitis virus (TBEV, family *Flaviviridae*) is medically the most important tick-borne virus in Europe gaining growing public health interest due to its increasing incidence. The clinical manifestations caused by TBEV range from asymptomatic infections and fevers, with complete recovery of patients, to debilitating or fatal encephalitis. TBEV is associated with *Ixodes ricinus* (European TBEV subtype) and *I. persulcatus* (Siberian and Far-Eastern TBEV subtypes) ticks. In nature, TBEV is maintained in a cycle involving ticks and wild vertebrate hosts, particularly small rodents. Although an effective vaccine against TBEV is available, the increasing incidence of human TBEV infections indicates that the current preventive measures are not sufficient. Targeting TBEV in frame of the efforts to develop universal transmission-blocking anti-tick vaccine should be therefore considered as a promising approach to decrease TBEV infection risk.
In frame of the ANTIDotE project, aimed to find anti-tick vaccines protecting against multiple human tick-borne diseases, we performed comparative transcriptome analysis of *I. ricinus* nymphs in the context of TBEV infection and feeding. The nymphs were first infected by TBEV by transcoxal inoculation and then allowed to feed on laboratory mice.

The nymphs were collected at several time-points during feeding and dissected in order to extract RNA from their salivary glands. Data obtained by the massive analysis of cDNA ends (MACE) method indicate that the tick feeding rather than TBEV infection is the main driving force of the tick differential gene expression. Nevertheless, several genes specifically upregulated in the salivary glands of either the TBEV-infected nymphs or feeding nymphs could be identified and are further evaluated as putative anti-tick vaccine candidates.

TBEV-13
Habitat suitability of the TBE virus in Central Europe

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Each year, more than 2,000 human tick-borne encephalitis (TBE) cases are confirmed in Europe, a remarkable proportion outside official risk areas or know endemic areas. The population in non-risk areas increasingly ask, whether they should consider a vaccination against TBE. Available risk maps, like those of the Robert Koch-Institute in Germany, are based on registered human TBE cases. Alternatively, the risk of being bitten by a TBE virus infected tick (exposure-based approach) can be estimated with a species distribution model. Contrary to human TBE case-based risk assessment, the spatial risk is estimated based on virus detections in ticks and animal hosts. Therefore, a Random Forests model based on decision trees was implemented and applied to a dataset comprising more than 800 georeferenced TBE virus records in ticks and animal hosts. As environmental predictors temperature- and precipitation-dependent bioclimatic parameters of the Worldclim dataset as well as land use and coverage of the GlobCover dataset were applied. With the Random Forests model the potential TBE virus distribution in Central Europe is estimated. Beside in known endemic areas, high habitat suitability is also shown in northern Germany, the Netherlands, and Belgium. This novel map can support decision makers to identify risk areas for human TBE infections.

TBEV-14
Tick-borne Encephalitis Associated with Consumption of Unpasteurised Goat Milk in Podlaskie Voivodeship in June 2017 – a case series

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Tick-borne encephalitis virus (TBEV), belonging to the Flaviviridae family, remains one of the most frequent causes of central nervous system infections in Europe.[1] Between 2000 and 2015 in Poland were reported 3662 TBE cases, what resulted in incidence rate from 0.33 to 0.92/ 100,000 persons. Out of those, 45% of the cases were registered in Podlaskie Voivodeship in the northeastern part of Poland, which is well defined as endemic area of TBE in Poland.[2] The main transmission route for TBEV is by tick bites, but TBE can be also acquired by consumption of unpasteurised milk products.

The outbreaks of milky epidemic of TBE in Poland were documented in 1975, 1995 and 2014.[3,4,5] The aim of this report is to underline the risk of TBEV transmission by consumption of unpasteurised dairy products and to present clinical cases of 4 patients, who developed TBE as the result of ingestion of raw goat milk
A retrospective analysis of 4 case histories of patients aged 24 to 36 years who had been hospitalised in the Infectious Diseases Ward of General Hospital in Hajnówka and in the Department of Infectious Diseases and Neuroinfection of the Medical University of Białystok between 10th June and 14th July 2017. TBE was diagnosed based on clinic and epidemiological data and confirmed by serology (ELISA) All of the patients were monks and they domiciled in monastery. All four patients were on vegeterian diet, in which the source of protein was the consumption of raw goat milk. None of the patients had a history of vaccination against TBEV. Cases were characterised by a mild, biphasic course. Patients had influenza-like symptoms with concomitant fever and presented no signs of meningitis. The blood and CSF examination in all patients were consistent with the TBE. Persons in the TBE-endemic areas should consume only boiled milk and be vaccinated. There is a need to enhance public awareness through educational campaigns about the possibility of transmitting TBEV by milk products.

TBEV-15
New areas of TBE incidence in Poland as result of intensification of surveillance.

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Tick borne Encephalitis (TBE) has been under mandatory reporting since 1970 in Poland. Geographical distribution of TBE human cases in Poland is unequal. North-Eastern part of Poland (Podlaskie region) is considered as endemic with highest incidence, and in this region serological tests are routine. In other regions of Poland it is not possible to detect the antibodies against TBE in the serum (or cerebrospinal fluid), the presence of which confirms the diagnosis. This results in the ICD-10 diagnosis of A87 (viral meningitis) instead of A84 (tick-borne viral meningitis), which may affect the significant underestimation of cases occurring in Poland.
The aim of the study is increase of detecting TBE cases by way of systematic serological testing for TBE (active part of surveillance) in all subjects with neurological infection of likely viral etiology reported in the 20 chosen hospitals. This is designed as a multicenter, prospective study. Subjects diagnosed with neuroinfection of unknown etiology, hospitalized in selected voivodships, are offered free of charge diagnostic testing for TBE (Virotech), as a part of the routine evaluation with dedicated questionnaire. A cases of neuroinfections of unconfirmed etiology are enrolled to the study. In 2018 were reported less cases of TBE than in 2017 (228 vs 161). During 5 months of the study, of 212 samples, 17 cases of TBE are confirmed. Some positive samples were from areas of low incidence, and some in quite new areas. Despite lower incidence of TBE in 2018 in Poland and 5 months of the study, new areas of TBE prevalence are detected.

Preliminary results suggest that TBE cases are underdiagnosed and prevalence of TBE in Poland is underestimated.The newly formed surveillance network for TBE should be active nature through the involvement of provincial and district hospitals from endemic and non-endemic area of Poland. Educational and preventive activities, verification of previously existing risk maps should be performed.

TBEV-16
First phylogenetic analyses of TBE virus detected in Lower Saxony

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Background and objectives: Tick-borne encephalitis (TBE) is the most important tick-borne arboviral disease in Europe. To date, the main endemic regions in Germany are located in the southern half of the country. Recently, sporadic human TBE cases were reported outside of these known endemic regions. Detection and characterization of invading TBE virus (TBEV) strains will considerably facilitate the surveillance and assessment of this important disease
Materials & methods: Ticks were sampled in 2018 in several locations of Lower Saxony associated with human cases and/or human sero-positivity. Ticks were pooled according to stage and sex. Testing for TBE viral RNA was done using the RT-qPCR (Schwaiger & Cassinotti 2003). In positive pools the E gene was amplified and sequenced for phylogenetic analysis.
Results: A total of 4797 ticks were sampled and tested. Five positive pools could be detected in the areas of “Rauher Busch” and “Barsinghausen/Mooshütte”. The whole E genes (1488 nucleotides) of the two TBE virus strains could be amplified and analysed. According to these data, the two virus strains are closely related to each other and cluster genetically with a TBE virus from Poland isolated in 1971.
Conclusion: This study provides for the first time data on the phylogeny of TBE virus in Federal State of Lower Saxony. The phylogenetic data imply that closely related TBE viruses are circulating in the two locations and that the origin of the TBE virus strains may originate from Poland. These results strengthen the hypothesis of an east-west invasion of TBE virus.

TBEV-17
Competence of the vector restricting tick

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Tick-borne encephalitis virus (TBEV), mainly transmitted via the tick vector *Ixodes ricinus,* causes one of the most important central nervous system (CNS) viral disease which is endemic in 27 European countries. In Germany, 583 human cases have been registered in 2018. Risk areas of TBEV are mainly located in the south of Germany, despite the nationwide distribution of the vector. For instance, the federal states Bavaria (224 cases) and Baden-Wuerttemberg (271 cases) reported the highest incidence rates in the last years as compared to Lower Saxony (8 cases, 2018). Reasons for the enhanced prevalence in the southern parts of Germany are still unexplained. Specific adaptation of tick populations to TBEV isolates and the resulting vector competence of those populations for TBEV is completely unexplored but may explain at least some of the differential distribution of endemic foci. For investigation of possible population-based differences, ticks of two different areas in Germany (Lower Saxony and Bavaria) were infected via an *in vitro* feeding system. Infection dose was 1x 106 FFU/ml of a TBEV isolate obtained from the endemic foci Haselmühl. Viral RNA was detected using qRT-PCR protocol by Schwaiger & Cassinotti. Groups were compared regarding tick origin, ticks" body part as well as the post infection incubation period. Concerning tick feeding, findings suggest a shift in tick activity. Ticks of TBEV endemic foci Haselmühl tend to be more active in May and October, whereas ticks from Hannover seem to be more active in July and August. Significant differences in feeding rates (May: Haselmühl 43.81% engorged ticks, Hannover 19.44% and August: Haselmühl 33.13%, Hannover 73.08%) could be shown between the two locations. Results of the qRT-PCR detecting viral genomes showed higher TBEV infection rates in ticks from the Haselmühl foci. Viral loads differ equally between the groups with ticks from Haselmühl having generally higher viral RNA loads and higher variances in viral loads than ticks from Hannover.
Taken together, our findings suggest a specific adaptation of the Haselmühl tick populations to the respective Haselmühl TBEV virus isolate. This is in line with the observation that TBEV endemic foci usually remain stable for extended periods regarding location, prevalence and stability of the viral sequences. However, this study currently only analyzed the relationship of virus isolate and two tick populations during one year. For verification of the proposed specific adaptation, more experiments need to be conducted during the tick season (April-October) in the next years and further virus isolate-tick population pairs need to be taken into this analysis.

POSTER ABSTRACTS

Tick-borne pathogens | TBP-1 – TBP-37

TBP-1

Molecular Identification and Characterization of *Theileria* spp responsible for Ovine Theileriosis in Egypt

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Introduction: Tick-borne hemoprotozoan parasites like *Theileri*a spp is among the most economically important infections of small ruminants all over the world especially in tropical and subtropical regions. *T. lestoquardi and T. ovis* are the main cause of ovine theileriosis (Guo et al., 2002; El Imam et al., 2015). In Egypt there is no enough data about the molecular characterization of these species especially in Upper Egypt and Egyptian Oases.
Objective: This study was conducted for molecular identification and characterization of *Theileria* spp of sheep in three different governorates in Egypt (Menoufia, Behira and EL-Wady EL-Geded).
Materials & methods: Study was conducted on 115 sheep from different ages and sex belonged to three different governorates in Egypt (Menoufia, Behira and EL-Wady EL-Geded). Blood samples were collected from each animal. The extracted DNA from sheep blood were subjected to PCR using primers targeting 18S ribosomal RNA gene; followed by sequencing and phylogenic analysis (AL-Hosary et al., 2015; Elsify, et al., 2015).
Result: Out of these 115 samples six samples were confirmed positive by PCR for 18s rRNA gene. the nucleotide blast of the generated sequences revealed that five sequences were *theileria ovis* from Egyptian Oases and Menoufia governorate all of these samples were submitted to Gene Bank under the following accession number (KY494648, KY494649 KY494650, AB986193 & AB986194) and only one sample was *theileria lestoquardi* (KY494651) from Egyptian Oases.
Conclusion: This paper considers the first molecular report of *theileria ovis and theileria lestoquardi* in sheep in Egypt accompanied with phylogenic analysis. This infection is one of the destructive obstacles for sheep production in Lower Egypt and Egyptian Oases and need more investigation in the future to evaluate its epidemiological situation and construct plans for eradication.

TBP-2

Genome sequencing of a British ovine isolate of *Anaplasma phagocytophilum* and proteomic analysis of p44 expression in tick cells *in vitro*

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Introduction: Our understanding of the cell biology and pathogen-vector interactions of *Anaplasma phagocytophilum (Ap)* have been shaped largely by analysis of zoonotic strains isolated in the USA. However, human granulocytic anaplasmosis is very rare in Europe, whereas tick-borne fever caused by distinct *Ap* strains in ruminants is widespread. There is an urgent need to understand the differences in biology and epidemiology between North American and European *Ap* strains.
Objectives: To address this deficit, we aimed to sequence the genome of a British ovine isolate of *Ap* and to examine its expression of the p44 outer membrane protein family (which undergoes antigenic variation) during infection of tick cells *in vitro*.
Materials & methods: We sequenced the genome of an *Ap* isolate (Old Sourhope, OS) from a Scottish sheep using Illumina technology. Genome annotation was undertaken with a special focus on the p44 protein-coding genes. Preliminary experiments and predicted protein sequences from the genome were used to design parallel reaction monitoring (PRM) assays for quantification of peptides from ten p44 proteins by mass spectrometry. *In vitro* growth of the pathogen was performed in the *Ixodes scapularis* cell line ISE6.
Results: The OS genome was recovered in 268 contigs and annotation of protein-coding genes revealed 138 p44-like sequences. Of the ten p44 proteins prioritised for PRM assays, eight were detectable, but a single variant was quantitatively highly dominant. The orthologues of this dominant p44 protein were found mainly in the published genomes of other ruminant (but not human, equine or canine) strains of *Ap.* Comparison with published data from OS in experimentally-infected sheep revealed no overlap between the p44 profiles expressed in tick cells compared with sheep *in vivo*.
Conclusion: The p44 proteins of *Ap* may be involved in determining host specificity. Moreover, distinct p44 variants may be expressed in the tick compared with the mammalian definitive host.

TBP-3

The assessment of the risk of *Coxiella burnetii* and *Rickettsia* spp. infections in north-eastern Poland

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Introduction: In the Polish medical literature, a few cases of *Coxiella burnetii* and *Rickettsia* spp. infections were described. Not much is known about the occurrence of these rare pathogens in Podlaskie voivodship, which is known as endemic area for tick-borne diseases.

Objectives: The main aim of the study is to assess the occurrence of anti- *Coxiella burnetii* and *Rickettsia* spp. antibodies in the inhabitants of the Podlasie Voivodeship. Another goal is to assess the risk of symptomatic infection with *Coxiella burnetii* and *Rickettsia* spp.
Materials & methods: The study consised of two parts:
1. Evaluation of the anti- *Coxiella burnetii* and *Rickettsia* spp. antibodiesin 184 inhabitants of the Podlasie Voivodship exposed to ticks. Patients were divided into three groups: Ia-82 healthy foresters, IIa – 82 farmers, IIIa - - 20 blood donors. The antibody titers were determined by ELISA:
2. Rickettsia IgG (spotted Fever Group) ELISA (EIA-5297; DRG International Inc. USA) 2. Coxiella burnetti (Q-fever) Phase 1 IgG ELISA (DRG International Inc. USA)
3. Estimation of the occurrence of genetic material (by means of PCR) of *Coxiella* spp. and *Rickettsia* spp. in 540 patients hospitalized due to various symptoms after tick-bite (Group Ib). The control group consisted of 20 blood donors (Group IIb).
PCR was performed using:
4. Hum PCR Coxiella burnetii detection kit (Bioingentech Ltd., Chile)
5. PCR Rickettsia spp. Detection kit (Bioingentech Ltd., Chile).
Results: 6. In Group Ia and IIa the presence of IgG anti- *Rickettsia* spp. antibodies was signific antly more frequent (57% vs 27%) than in Group IIIa (0%) ($p<0.05$). 2. Only in Group IIa, anti- *Coxiella burnetii* antibodies in the IgG class (4%) were detected.
7. No genetic material of *Coxiella burnetii* or *Rickettsia* spp was found in Ib and II b Groups.
Conclusions: Inhabitants of Podlaskie Voivodship are exposed to *Coxiella burnetii* and *Rickettsia* spp infections, however it rarely leads to a symptomatic infection.

TBP-4
Emerging pathogens in questing *Ixodes persulcatus* in Karelia (Russia)

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Ixodes ricinus and *I. persulcatus* ticks are the primary vectors of pathogens that cause dangerous tick-borne diseases in northern Europe. In recent years, notable changes in the abundance and distribution of these species have been observed, including an expansion of their sympatric range, primarily due to the more dangerous vector *I. persulcatus*. This report presents the results of long-term studies of the prevalence of the main tick-transmitted pathogens in Ixodes persulcatus ticks conducted in Karelia since 2007. Adult questing ticks were collected during field visits in May–June by flagging from vegetation. A total of 1321 adult ticks from different parts of Karelia were studied. As a result of genotyping, tick-borne encephalitis virus (Siberian subtype), *Borrelia afzelii*, *B. garinii*, *Ehrlichia muris* were detected. TBEV was detected in ticks from the most of the investigated area, including northern border of *I. persulcatus* range. Average TBEV prevalence in ticks was 2.2%. There were no significant annual differences in the occurrence of the virus. Virus prevalence in *I. persulcatus*

females and males was 2.6% and 1.5%, respectively. Average infection rate of ticks with *Borrelia burgdorferi* s.l. was 29%, and varied from 7 to 78% depending on the sampling place and from 15 to 50% depending on the year of tick collection. Infection rate of *I. persulcatus* females and males was 25% and 16%, respectively. According to the results of multiple primers real-time PCR analysis (TBEV, *B. burgdorferi* s.l., *E. muris*, *A. phagocytophilum*) of 400 specimens of *I. persulcatus*, the proportion of uninfected ticks was 57%, infection rate of ticks with *Borrelia* sp. was 40.3%, with TBEV – 3.8%, and with *E. muris* – 2.7%. Co-infections were detected in 16 ticks (4%) in two combinations Borrelia-TBEV (8) and Borrelia-Ehrlichia (8). There is no determinismin occurrence of co-infections (the probability of their occurrence does not differ from random).

TBP-5
The role of different ungulate species in the ecology of tick-borne diseases.

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Introduction and objective: The incidence and societal burden of tick-borne diseases is rising due to increasing tick numbers, caused by changing weather, vegetation and host populations. There is therefore a strong need to develop and/or identify mitigation measures to reduce the number of infected ticks in the landscape. Ungulates play a central role in the life-cycle of ticks and their management is one proposed intervention strategy. However, there are knowledge gaps concerning the effect of different ungulate species on the ecology of tick-borne diseases. Here, we present a research approach to fill some of these knowledge gaps.
Materials & methods: We look at different pathways in which Swedish ungulates (four deer species and wild boar) might influence ticks and two tick-borne pathogens: *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum*. We aim to identify 1) the direct impact of difference in morphology and movement behavior of the ungulate species on tick load and tick-borne pathogen infection and 2) the indirect effects of ungulate species on ticks and tick-borne pathogens through interactions between ungulates, rodents, and vegetation. We will present some initial data from a study where we collected ticks and spleen samples from shot fallow deer, roe deer, red deer, moose and wild boar in South-Central Sweden. In the same area, we also collected questing ticks. All ticks and tissue samples are tested for the presence of *A. phagocytophilum* and *B. burgdorferi* s.l.
Results: Preliminary results show that red deer had the highest tick load (larvae, nymphs and adults combined), followed by roe deer, fallow deer and moose. Wild boar had negligible tick loads. These preliminary results indicate that tick loads vary among ungulate species and that these species thus likely play very different roles in the ecology of tick-borne diseases.

TBP-6
First report on Q fever cases in patients from Novosibirsk region, Western Siberia, Russia

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Introduction: Q fever is a widespread zoonotic disease caused by *Coxiella burnetii*, an intracellular bacterium infecting humans and vertebrates. Domestic ruminants are believed to be the main reservoir. Transmission is primarily but not exclusively airborne, and ixodid ticks usually act as vectors. Despite the obligatory registration of Q fever in Russia, where its average incidence rate is about 0.1 per 105 people, the disease has not been registered in patients of the Novosibirsk region of Western Siberia, Russia before.
Objectives: The aim of our study was to identify the causative agent and describe the clinical manifestations of Q fever in patients hospitalized in the City Infectious Clinical Hospital No.1, Novosibirsk, Russia.
Materials & methods: Blood of 325 patients hospitalized after tick"s bite and/or visiting the forest park areas in May-July, 2018 was tested for the presence of tick-borne encephalitis and West Nile viruses, *Borrelia burgdorferi* s.l. and *B. miyamotoi*, *Rickettsia* ssp., *Francisella tularensis, C. burnetii, Babesia sp., and Bartonella spp. by real-time PCR. G*enetic study of *C. burnetii* was performed with primers to heat shock protein B (htpB) gene and repetitive element IS1111.
Results: DNA of *C. burnetii* was detected in five blood samples, that was confirmed by sequences of two studied loci. None of other pathogens were found in the patients" blood.
Tick"s bite/crawling were noted in the anamnesis of three patients. Clinical manifestations were represented by the acute onset of the disease, febrile syndrome, pharyngitis, weakness, fatigue, moderate severity. In four patients, the temperature reached 39-40°C. Hepatitis and hepatomegalia were observed in two patients. In one patient with immunodeficiency caused by Crohn's disease and GC and cytostatics treatment, the disease had severe form with the development of meningoencephalitis and pneumonia. The duration of the febrile period was 7-10 days in the course of treatment.

TBP-7
Implementation of the DAMA protocol: mitigation of infection risk with emerging tick-borne zoonotic bacteria in cities

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Tick-borne diseases represent major public and animal health issues worldwide. Urban environments have special ecological characters in the complex communities of pathogens, ticks and hosts. From

a public health perspective, city parks and peri-urban recreational areas are typical meeting places for humans and ticks. Ticks in this respect serve as a bridge for pathogens connecting reservoir hosts with humans. Here we attempted to apply the general protocol, DAMA (documentation–assessment–monitoring–action), which is an integrated proposal to build a proactive capacity to understand, anticipate, and respond to the outcomes of accelerating environmental change. We have chosen hedgehogs, one of the most successful urban adapters reaching up to nine times higher densities in urban than in rural areas leading to an unbalanced vertebrate community that easily provides favourable ecological conditions for ticks and pathogens.We carried out an eco-epidemiological study of an urban population of the Northern white-breasted hedgehog (*Erinaceus roumanicus*) in a park of Budapest for 3 years. Hedgehogs were live-captured and anesthetized; DNA was extracted from ear tissue samples and molecular analyses were performed. Surprisingly 216/230 (94%) ear tissue samples were positive for the Lyme Borreliosis spirochete. Prevalence of *B. burgdorferi* sensu lato in ticks flagged in the park was also high, 33.15% (177/534). Sequencing identified *B. afzelii, B. spielmanii* and *B. bavariensis* in hedgehogs and in questing ticks. Two other human pathogens, *Candidatus* Neoehrlichia mikurensis (2.3%) and *Anaplasma phagocytophilum* (76%) were also detected in the hedgehogs and with 22.1% and 19.29%, prevalence in questing ticks, respectively. Our data show that documentation, assessment and monitoring of ticks and reservoir hosts is crucial and we also make suggestions about possible actions, e.g. tick awareness, installing public toilets to avoid visiting high-risk habitats within city parks.

TBP-8

Translational biology of tick-borne diseases: flavivirus infection of tick *ex vivo* organotypic cultures and applications for disease control

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The Lyme disease tick vector, *Ixodes scapularis*, transmits a number of pathogens, including tick-borne flaviviruses (TBFVs). TBFV infections cause thousands of human encephalitis cases worldwide each year. In the US, confirmed human infections with TBFV Powassan virus (POWV) are increasing and have a fatality rate of 10-15%. In addition, Langat virus (LGTV) is often used as an experimental model TBFV of low neurovirulence. Currently, the detailed characteristics of TBFV replication and dissemination within tick organs are poorly characterized and are important due to the relevance of organs being barriers to infection that affect downstream transmission. A deeper understanding of

virus biology in the tick vector may inform effective countermeasures to reduce TBFV transmission. The overall goals of this work were 1) to develop short-term, *ex vivo* organ cultures of the midgut (MG) and salivary glands (SGs) from the female *I. scapularis* tick, 2) to examine initial TBFV replication and spread in individual organs, and 3) to utilize the organ cultures for dsRNA-mediated RNA interference (RNAi) assays to identify impact on TBFV replication. Organs were dissected from unfed and fed female ticks and cultured *ex vivo*. The organotypic cultures were metabolically active for up to 8-10 days and supported TBFV growth. Specifically, confocal microscopy of organs infected with green fluorescent protein-tagged LGTV demonstrated LGTV replication and spread. Furthermore, immunohistochemistry confirmed LGTV envelope and POWV protein expression and spread within the infected organ cultures. Infectious LGTV and POWV were produced from infected organ cultures. Using transmission electron microscopy, we also identified cellular localization of LGTV particles in infected MG and SG cultures of unfed female ticks. Thus, the *ex vivo* organ cultures were a suitable system for study of virus replication in individual organs. In order to apply these organ cultures to identify tick genes as possible targets for countermeasure development, we first identified an active RNAi response in the organ cultures. Transfection of TBFV-infected MG and SG cultures with dsRNA specific to the LGTV 3"UTR reduced infectious LGTV/POWV replication, providing a proof-of-concept use of RNAi in *I. scapularis* organ cultures. In ongoing work, we have completed RNAi assays in SG cultures with dsRNA specific to tick transcripts and have identified a reduction in infectious POWV replication with a select number of tick transcripts. The results of this study provided novel information on TBFV replication and spread in specific cell types using *ex vivo I. scapularis* tick organs. This system may prove useful as a translational tool for identifying potential tick transcripts/proteins that can be used as targets for TBFV countermeasures. This research was supported by the Intramural Research Program of the NIH, NIAID.

*Relevant publications that involved and/or discussed this research:

- Grabowski JM et al. 2019. *mBio*. In press.
- Grabowski JM, Offerdahl DK and Bloom ME. 2018. *ACS Infectious Diseases*. 4(3):247-256.
- Grabowski JM and Hill CA. 2017. *Frontiers in Cellular and Infection Microbiology*. 7:519.
- Grabowski JM et al. 2017. *mBio*. 8: e01255-17.

TBP-9

Case of *Dermacentor*-borne necrosis erythema lymphadenopathy (DEBONEL) imported from Spain

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We report a case of a German nine-year old girl that was bitten by a *Dermacentor marginatus* tick in Andalusia (Spain) during holidays in October 2018. Returned to Germany she developed fever of 39°C, painful lymphadenopathy, local necrosis and eschar at two sites of her head, mild elevation of erythrocyte sedimentation rates C-protein and other liver enzymes levels. After hospitalization she received an intravenous therapy with ampicillin. After three days the fever decreased. Meanwhile we received the tick and eschar of both biting sides. We tested tick and eschar for Rickettsia spp, *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum, Babesia spp by molecularbiological*

methods. All tests were negative except the test for Rickettsia. We revealed and classified the Rickettsia in the tick by molecularbiological *means* To confirm our findings we investigated the eschar with microscopic techniques. After being discharged the girl was additionally treated ten days by doxycycline and recovered completely.

TBP-10

Molecular detection and characterization of *Rickettsiae* in ticks and mites collected from small rodents in Curonian Spit, Lithuania

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The genus *Rickettsia* comprises obligate intracellular gram-negative bacteria, of which many cause human infections in all continents inhabited by humans in the world. Rickettsiae are characterized by complex life-cycles and diversity of hosts and transmission strategies. The arthropod vectors are important in the natural maintenance of rickettsial pathogens. Information on the circulation of Rickettsiae and their association with different arthropod vectors in the Baltic countries is scarce. The aim of our study was to investigate the prevalence and diversity of *Rickettsia* spp. in ticks and mites parasitizing different species of rodents in the Curonian Spit of Lithuania. A total of 238 rodents of six different species were collected. Rodents were found to be infested with *Ixodes ricinus* ticks (n=596) and 5 species of parasitic mites (n=550) from Laelapidae family (*Laelaps agilis*, *Hyperlaelaps microti*, *Haemogamassus nidi*, *Eulaelaps stabularis*, *Myonyssus gigas*). *Rickettsia* DNA in ectoparasites was detected using a nested PCR that targeted partial *gltA* gene. Identification of *Rickettsia* species was based on sequencing and phylogenetic analysis of *17kDa* and *gltA* genes. Infection rates were calculated as the maximum likelihood estimation (MLE). *Rickettsia* DNA was detected in *I. ricinus* larvae and nymphs and in for mite species. *Rickettsia*-positive *I. ricinus* ticks were found on *Apodemus flavicollis*, *Myodes glareolus*, *Micromys minutus*, *Microtus oeconomus* and *M. arvalis* rodents. Infected mites were collected from *A. flavicollis* and *M. minutus*. The infection rate was found higher in *I. ricinus* ticks (MLE = 26.5%) compared with mites (MLE = 9.3%). Mites feeding on rodents harboured *R. helvetica*, *R. felis* and unidentified *Rickettsia* sp. In *I. ricinus* only *R. helvetica* was detected. To our knowledge, this is the first report of the occurrence and molecular characterization of *Rickettsia* spp. in ticks and mites feeding on different rodent hosts from the Baltic countries.

TBP-11

Epidemiological investigation of Crimean-Congo Hemorrhagic Fever Virus foci among Livestock in the endemic region of Pakistan

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic disease caused by the arbovirus Crimean-Congo hemorrhagic fever virus (CCHFV). In 2016, a cross-sectional study was conducted in the Balochistan province of Pakistan. Blood samples were collected from 1600 sheep and goats in Quetta, Sibi and Zhob divisions of Balochistan. In the molecular analysis (RT-qPCR) 8 (5%, CI: 2%-10%) out of 160 sheep serum pools were positive for the CCHFV genome fragments, while all goat serum pools (0%) were negative in this assay. In the serological analysis, 149 (19%, CI: 16%-21%) out of 800 sheep serum samples and 37 (5%, CI: 3%-6%) out of 800 goat serum samples were positive for CCHFV-specific IgG antibodies. In Zhob division 53% (41 out of 77, 95% CI: 42%-65%) farms were positive for the CCHFV-specific IgG antibodies. Similarly, in Sibi division 48% (27 out of 56, 95% CI: 35%-62%) farms and in Quetta division 48% (13 out of 27, 95% CI: 29%-68%) farms were positive for CCHFV-specific IgG antibodies. The open type of housing (OR= 3.76, CI: 1.57-9.56, p= 0.003), grazing (OR= 4.18, CI: 1.79-10.37, p=0.001), presence of vegetation in/around the farm (OR= 3.13, CI: 1.07-10.15, p=0.043), lack of treatment against ticks (OR=3.31, CI: 1.16-10.21, p=0.029), absence of rural poultry (OR=2.93, CI: 1.41-6.29, p-value=0.004), sheep with age $\geq$ 2 years (OR=2.72, CI: 1.36-6.22, p=0.008), sheep infested with ticks (OR=2.11, CI: 1.38-3.29, p<0.001), and goats infested with ticks (OR=2.68, CI: 1.23-6.72, p=0.02) were identified as statistically significant risk factors associated with the occurrence of CCHFV infections in livestock. The seroprevalence was higher in the northern part of the province. The risk factors identified in this study might elevate the probability of CCHFV infection in livestock and most importantly humans who are in close contact with these animals may consequently also at a higher risk of infection with this virus.

TBP-12

Investigation of the Role of Tortoises and *Hyalomma aegyptium* Ticks in Crimean-Congo Hemorrhagic Fever Epidemiology

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Since CCHF was first detected in Turkey in 2002, it caused over 10,000 human cases and 500 deaths. Although cases were reported from all parts of the country, the northern parts of the Central Anatolia and Eastern Anatolia are the endemic regions, where the vast majority of cases are seen. Geographic

risk determinants for density of cases are mainly virus prevalence in tick vectors, seropositivity in humans, and susceptible animal populations.
Unexpectedly only 30 cases and 3 deaths were reported in the Thrace region of Turkey, although human seroprevalence there is similar to the endemic region and a higher percentage of the tick population in Thrace is CCHFV positive, as compared with the epidemic region. The remarkably lower number of cases in Thrace suggests that virus-, vector-, and/or host-dependent variables may explain the divergence in this region. A putative explanation for the dissimilarity could be the presence of two different CCHFV genotypes (Europe1 and Europe2) in Thrace whilst only one type is dominant in Central Anatolia. On the other hand, the genotype prevalent in Central Anatolia (Europe1) is also widely distributed in ticks in Thrace region. The most significant difference between these regions is that *H. aegyptium* is the dominant tick species collected from human-biting cases in Thrace, while it is *H. marginatum* in endemic region. The potential role of *H. aegyptium* in CCHFV transmission has not been examined previously. Guided by this evidence, the present study was designed to investigate whether *H. aegyptium* or tortoises (the dominant host for *H. aegyptium)* play a role in the natural cycle of CCHF by evaluating if CCHFV can be detected in them.
Blood and tick samples were collected from tortoises found in natural areas and cemeteries, in their active seasons between May 2017 and September 2018. Sample collection was intensified around 25 foci where CCHF cases were reported previously. A total of 127 tick pools (both questing and from tortoises) and 21 tortoise blood samples were tested for CCHFV by nested RT-PCR. CCHFV RNA was detected in 10 out of 127 tick pools (7.9%) and in 2 out of 21 tortoise blood samples (9.5%). Positive ticks were derived from 9 tortoises out of 42. Virus was also detected in 2 questing tick pools. A sequenced isolate was found to be similar to Kosovo-Hoti strain, based on CCHFV S segment genotyping. This study suggests that tortoises and their ticks may to play a role in the epidemiology of CCHF in the Thrace region. Future research will definitively determine the vector competence of *H. aegyptium* for CCHFV (either or both genotypes), as well as the competency of tortoises as reservoirs for CCHFV.

TBP-13
Abundance of ticks and tick-borne bacterial pathogens in northwestern Germany

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Ticks from the genus *Ixodes* are of importance for public and animal health because of their vector function for pathogens. The present study investigates the abundance of ticks and tick-borne pathogens in different areas in northwestern Germany.
To analyze tick abundance, an area of 100 m^2 was flagged monthly at three locations for each region of Bremen, Emsland, Hanover, Kassel and Uelzen from February to November 2018. Furthermore, from April to October 2018, 15 additional ticks (5 females, 5 males and 5 nymphs) were collected monthly at each site (1,575 ticks in total) to investigate *Borrelia* spp., *Rickettsia* spp. and *Anaplasma pagocytophilum* prevalence by qPCR.

The average observed abundance of ticks in 2018 ranged from 2.1 collected ticks/100 m^2 in an urban area in Kassel to 67.1 ticks/100 m^2 in a mixed forest in Emsland. Of the total 3,910 ticks, 96.5% were identified as *I. ricinus*, 3.3% as *I. inopinatus* and 0.1% as *I. frontalis*, while 8 could not be assigned to any species yet. Of the ticks investigated by qPCR, 31.6% (497/1575) were infected with *Borrelia* spp., 35.4% (558/1575) with *Rickettsia* spp. and 8.0% (126/1575) with *A. phagocytophilum*, while 41.8% (659/1575) were not infected. The regional prevalence of ticks infected with *Borrelia* spp. ranged from 23.8% (75/315) in Bremen to 40.9% (129/315) in Hanover, infection with *Rickettsia* spp. ranged from 27.3% (86/315) in Emsland to 44.7% (141/315) in Hanover and *A. phagocytophilum* infection ranged from 5.7% (18/315) in Emsland and Kassel to 13.8% (43/315) in Uelzen. Regarding infection rates in different developmental stages, 60.0% (630/1050) of adult ticks and 54.9% (288/525) of nymphs were infected with at least one of the investigated pathogens. The present study showed regional differences in tick infection rates in northwestern Germany. As more than 50% of ticks were infected with a zoonotic pathogen, it is important broaden our knowledge on the geographic distribution of ticks and associated pathogens.

TBP-14

Development of an *in vitro* feeding system for ticks as tool to explore the potential transmission routes of Q fever

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Coxiella burnetii is an obligate intracellular bacterium that causes the disease Q fever in a wide range of hosts including humans. The main known transmission route of this zoonotic agent is the inhalation of contaminated aerosols.
Q fever is also considered as vector borne disease, since *Coxiella*-DNA could be detected in ticks in many regions worldwide. However, recent research on the tick microbiome revealed a high prevalence of *Coxiella*-like bacteria in ticks. These endosymbionts are assumed to be non-pathogenic although having a close genetic relationship to *C. burnetii*. Taking into account that commonly used target genes for PCR detection of *C. burnetii* in ticks may have caused misidentification previously; the role of ticks in Q fever transmission remains ambiguous.
The goal of the project is to evaluate if ticks are capable of transmitting *C. burnetii* via feces or saliva. Therefore, we adapted silicone-membrane based feeding chambers for the artificial feeding of adult *Ixodes ricinus* with uninfected or bacteria-spiked blood. Ticks as well as feces were tested at different time points during and after feeding using quantitative realtime PCR. *I. ricinus* nymphs are infected with *C. burnetii* and left for molting to adults in order to examine transstadial transmission. Up to now, we were able to feed *I. ricinus* females (mean fixation rate 88% at day 3) and nymphs in this *in vitro* system until engorgement. First results indicate that *C. burnetii* is excreted with feces during the feeding process on infected blood. However, this fecal transmission route needs to be further investigated as potential risk for infection by inhalation. Moreover, the reinfection process of infected ticks via saliva into uninfected blood will be examined to reassess the vector competence of *I. ricinus* as a potential reservoir for *C. burnetii.*

TBP-15
Trends of tick-borne pathogens in small mammal and ticks from Saxony, Germany

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Introduction: Rodents play an important role as hosts for immature tick life stages. Both, ticks and rodents, are of public health interest as they serve as reservoirs and vectors for tick-borne pathogens (TBP).
Objectives: The aim of this study was to reassess the prevalence of TBP in the city and surroundings of Leipzig and compare it with data from 2009-2014.
Materials & methods: In the years 2015-2017 rodents and ticks were collected in Leipzig, Saxony. Samples of rodents, attached and questing ticks were screened for the presence of *Anaplasma phagocytophilum, Babesia* spp., *Borrelia burgdorferi* s.l., *Candidatus* Neoehrlichia mikurensis (CNM) and *Rickettsia* spp. DNA of rodents was additionally tested for *Bartonella* spp. and *Hepatozoon* spp. Engorged larvae were processed in pools, other samples were tested individually.
Results: A total of 165 rodents (*Apodemus agrarius*, n = 1; *A. flavicollis*, n = 63; *Arvicola terrestris*, n = 1; *Myodes glareolus*, n = 137), 1256 parasitizing ticks (*Ixodes ricinus,* n = 1164; *Dermacentor reticulatus*, n = 92) and 577 questing ticks (*I. ricinus,* n = 547; *D. reticulatus,* n = 30) were collected. The prevalence in rodents was: 78.2% for *Bartonella* spp., 58.2% for CNM, 49.1% for *B. burgdorferi*, 29.1% for *Rickettsia* spp. and 24.2% for *Hepatozoon* spp. And in attached nymphs: 52.9% for *Rickettsia* spp., 13.5% for CNM and 11.3% for *B. burgdorferi*, while MIR for attached larvae was: 39.8%, 7.1% and 8.8%, respectively. *Babesia* spp. was detected only in questing ticks with prevalence 1.4%. And other TBP levels were as follows: 18.2% for *Rickettsia* spp., 7.3% for CNM, 6.4% for *B. burgdorferi*. All samples were *Anaplasma*-negative.
Conclusions: In 9-year long trend, amount and diversity of rodents and questing ticks have been declining. While the prevalence for *A. phagocytophilum* and *Babesia* spp. in general was decreasing, levels for CNM, *Bartonella* spp. and *B. burgdorferi* seemed to be rising. *Rickettsia* spp. has remained at the same level.

TBP-16
Canine breed predisposition in tick-borne diseases

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Introduction: Dogs are frequently infested by ticks, thus may be infected by tick-borne pathogens. Dogs service activities as well as size and coat structure may be the basis for tick exposure. In addition to that fact, breed associated immune competence may lead to breed predisposition for tick-borne diseases (TBD) in dogs.

Objectives: Literature review on canine borreliosis as well as current data on canine anaplasmosis and canine babesiosis, as well as serological data for tick-borne encephalitis, represent the basis for this report to calculate breed predispositions.
Materials & methods: For canine anaplasmosis (n=340) and canine babesiosis (n=450) breed data of infected dogs (proven by antigen or DNA detection) were compared to a population data set consisting of data from a dogs insurance company, a Viennese administration report, and canine patients of the Veterinary University Vienna (n=4.5 Mill). Breed data sets larger than 1000 (118 breeds) with a minimum of 5 cases per pathogen and breed were analyzed. For tick-borne encephalitis (n=177) breed data of infected dogs (proven by positive antibody titer or by antigen/ DNA detection) were included.
Results: An elevated breed risk (x times higher compared to the population based risk) for canine anaplasmosis was calculated as 16.9 for German Longhaired Pointer, 6.8 for Golden Retriever, and 7.8. for Airdale Terrier. An elevated risk for canine babesiosis was calculated as 10.9 for Alaskan Malamut, 7.7 for German Shorthaired Pointer, and 7.0 for Münsterländer. For TBE an elevated risk for seropositivity or disease was calculated as 15.9 for German Shorthaired Pointer, 14.9 for German Wirehaired Pointer, and 12.7 for Rottweiler
Conclusion: Common hunting dogs are at higher risk getting into contact with tick-borne pathogens. In other breeds, existing data on breed related immune system diversity lead to the conclusion that inbreeding may be another cause of breed predisposition for TBD.

TBP-17
First report of *Babesia microti* and *B. canis* in hedgehog blood

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Northern white-breasted hedgehog (*Erinaceus roumanicus*), a host for ecologically different I. *ricinus* and I. hexagonus, is considered to be involved in the maintaining ticks and tick-borne diseases in urban areas. They are reservoirs of tick-borne pathogens, including infectious agents of human disease, like *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and TBEV. However, there is limited knowledge about the periplasm infections; the only report concerns *Theileria* sp. detected in hedgehogs from China. The purpose of this study was identification of bacterial and protozoan agents in hedgehog's blood samples using next generation sequencing approach. Hedgehogs(100) were collected from city parks in Poznań in 2014-16. Each blood sample was tested for the presence of prokaryotic and eukaryotic DNA by amplification of V4 16SrRNA and D1 28SrRNA hypervariable regions, respectively. PCR products were sequenced using Ion Torrent PGMsystem. Additional screening was done using nested PCR and qPCR assays to confirm the NGS results. Using NGS approach, potentially pathogenic agents belonging to the genera previously known from hedgehogs were identified, e.g. *Anaplasma*(n=39), *Borrelia*(1), *Bartonella*(12), *Rickettsia*(32), and new for this host *Coxiella*(1). Sequence data of the D1 region let us to detect 7 samples positive for *Babesia*

microti; 5 of them were confirmed by qPCR assay, which failed for two other samples positive in NGS. False negative NGS and qPCR assays concerned samples with a very low copy number of the target template. Screening for *Babesia* DNA by the nested-PCR confirmed NGS results and revealed additional 4 samples positive for *B. canis* that have been missed in NGS due to mismatched D1 primers. Our results suggest that hedgehogs could be potential reservoirs of *B. microti*. Additional data from quantitative methods are needed to confirm infection by *B. canis*; however, the role of hedgehogs as potential reservoirs for piroplasms cannot be excluded. This study was supported by the Ministry of Science and Higher Education (grant no. N N304 325439)

TBP-18

Road-killed vertebrates as sentinel hosts for active surveillance of tick-borne pathogens

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Tick-borne zoonoses represent a serious threat for human and animal health. Thus, efforts are made to monitor the spatial distribution, intensity and diversity of their causative agents. Nevertheless, most of the studies focus on the tick vectors, whereas the vertebrate hosts remain neglected, mostly due to issues associated with complicated sampling (laborious, skill-demanding, legislative/species protection etc.). The main aim of our project is to verify whether carcasses of accidentally killed (mostly road killed) animals are suitable source of biological material for monitoring of selected tick-borne pathogens. Considering the epidemiological significance, we have focused specifically on urban habitats. Hedgehogs (*Erinaceus europaeus* and *E. roumanicus*), squirrels (*Sciurus vulgaris*) and blackbirds (*Turdus merula*) were chosen as representatives of wild animals that thrive in urban areas, are ordinarily infested by ticks and seem to be suitable hosts for many important zoonotic pathogens. A total of 169 specimens (75 hedgehogs, 22 squirrels and 72 blackbirds) were collected with the help of general public. Altogether 1 267 samples of different tissues were screened by multiplex qPCR assays. The prevalence of the main target pathogens reached for hedgehogs, squirrels and blackbirds respectively was 72%, 100%, 58% for *Borrelia burgdorferi* s.l., 89%, 82%, 51% for *Anaplasma phagocytophilum*, 21%, 77%, 3% for *Bartonella* spp. and 33%, 4%, 4% for *Rickettsia helvetica*. In conclusion, carcasses of accidentally killed vertebrates were confirmed as a useful source of biological material for monitoring of several tick-borne pathogens in urban environments.

TBP-19
Occurrence of tick-borne pathogens in Lithuanian Rodent communities

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Rodents represent an important group of mammalian hosts as considered reservoirs of tick-borne pathogens. However, *there still* is a *lack of knowledge* on the occurrence and diversity of tick-borne pathogens of zoonotic importance in rodents from Baltic countries. This study aimed to investigate the prevalence and diversity of tick-borne bacterial pathogens *Borrelia* spp., *Rickettsia* spp. and protozoan *Babesia* parasite in rodent communities.
Small rodents representing eight species - *Apodemus flavicollis*, *A. agrarius*, *Mus musculus*, *Micromys minutus*, *Myodes glareolus*, *Microtus oeconomus*, *M. agrestis* and *M. arvalis* were captured with live-traps in twelve different locations of Lithuania during 2013–2016. DNR was extracted from the spleen and urine bladder samples of small rodents. Real-time PCR and nested PCR were used to amplify different genome regions of *Borrelia* (*ospA* and 16S-23S ITS), *Rickettsia* (*gltA* and17kDa) and *Babesia* (18S rRNA) with subsequent sequence analysis. The overall prevalence of *Borrelia* spp. in rodent was 25%, *Rickettsia* spp. - 34.5 % and *Babesia* spp. – 6 %. *Borrelia* spp. were detected in six rodent species*, M. arvalis, M. agrestis, M. glareolus, M.oeconomus, A. flavicollis, A. agrarius*, *Rickettsia* spp. were detected in three species, *A. flavicolis, M. minutus,* and *M. glareolus,* and *Babesia* spp. – in *A. flavicolis, M. minutus, M. glareolus, M.agrestis* and *M.oeconomus.* All three pathogens were detected in *A. flavicollis* and *M. glareolus.* Sequence analysis of DNA from positive samples indicated the presence of two *Borrelia* species *Borrelia afzelii* and *Borrelia miyamotoi*, *Rickettsia helvetica* and *Babesia microti*. Coinfection with *B. afzelii* and *B. microti* was detected in *M.oeconomus.*

The findings of our study demonstrated the importance of rodent communities as considered reservoirs of these tick-borne zoonotic pathogens in Lithuania.

TBP-20
Geographically compartmentalized prevalence of *Theleria parva* in the African cape buffalo in Uganda correlates with distribution of the tick vector

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The protozoan *Theileria parva* is responsible for East Coast Fever (ECF), an important animal disease endemic to cattle across eastern, central and southern Africa. The infection and treatment (ITM) live vaccination method for control of ECF is increasingly being adopted, particularly in pastoral-

ist systems, but also in the small dairy sector. The method involves inoculation of animals with a potentially lethal dose of a tick stabilate containing live sporozoites and concurrent administration of a long acting formulation of oxytetracycline. However, it has recently been demonstrated that in some areas, such as central Kenya, *T. parva* derived from buffalo can break-through the immunity induced in animals immunized using the most widely deployed version of ITM. No comparable data exists for Uganda where only limited ITM vaccination trials have been performed in cross-bred or taurine cattle in the small dairy sector and interface areas have not been well characterized with regard to the risk of buffalo-to-cattle transmission of *T. parva*. There is limited evidence, from initial studies using reverse line blot, of regional differences in the prevalence of *T. parva* infections in buffaloes in Uganda and it is not yet known whether, and to what extent, vector distribution may have a role. We have confirmed the regional heterogeneity in the distribution of *T. parva* infected buffaloes in Uganda using the more sensitive *T. parva*-specific nested 104 kDa rhoptry antigen (p104) PCR assay, and serology using an indirect ELISA based on a recombinant version of the polymorphic immunodominant molecule (PIM). The combination of morphology-based species designations, mitochondrial cytochrome oxidase sequence typing and parasitised acini scoring demonstrated that geographical differences in prevalence of *T. parva* in buffalo in Uganda coincides with the distribution of the *R. appendiculatus* tick vector.

TBP-21

Turkey tick news: preliminary results from a molecular investigation into the presence of tick-borne pathogens in host-seeking ticks in Anatolia

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Introduction: Investigations to detect tick-borne pathogens (TBPs) in host-seeking ticks have the potential to provide highly valuable data.
Objectives: The aim of this study was to determine the presence of TBPs in host-seeking ticks obtained from 3 different ecological areas of Turkey.
Materials & methods: Host-seeking ticks were collected from the environment and identified according to morphological characteristics. DNA was extracted from each tick, and all ticks were screened for the presence of *Anaplasma* spp., *Babesia* spp., *Borrelia burgdorferi* sensu lato, and *Theileria* spp.
Results: In this study, 1012 ticks including 10 different species were collected from 47 collection points (3 provinces, 12 districts, 44 villages). Collected ticks were identified as *D. marginatus*,*D. reticulatus*,*H. aegyptium*,*H. excavatum*,*H. marginatum*,*Ha. parva*,*Ha. punctata*,*Ha. sulcata*,*I. ricinus*, and *Rh. turanicus*. PCR analyses identified that 27 ticks were infected by *Babesia* spp., 3 ticks by *Borrelia* spp. and 1 tick by *Theileria* spp. Partial *18S rRNA* sequences of *Babesia* spp.-positive samples revealed the presence of *B. occultans* in 12 *H. marginatum*, *B. crassa* in 9 *Ha. parva*, *B. rossi* in 3 *Ha. parva*, *Babesia* sp. tavsan1 genotype in 1 *H. marginatum*, *Babesia* sp. tavsan2 genotype in 1 *Rh. turanicus*, and an unclassified *Babesia* sp. in 1 *Ha. parva*. Phylogenetic analysis showed this potentially novel *Babesia* sp. to be located within the *Babesia* sensu stricto clade and closely related to domestic ruminant babesias. In addition, partial *fla* sequences of *Borrelia* spp.-positive samples revealed the presence of *B. afzelli* in 2 *I. ricinus* and *B. lusitaniae* in 1 *I. ricinus*. Finally, partial *18S rRNA* sequence of *Theileria* spp.-positive sample revealed the presence of *T. annulata* in 1 *H. excavatum*.

Conclusion: This study provides important data about both TBPs in host-seeking ticks in Turkey and a potentially novel *Babesia* sp. with a putative vector and new pathogen-vector species associations.

TBP-22
Candidatus Neoehrlichia mikurensis on the Western Seaboard of Norway. A coldspot within a hotspot.

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We have investigate the prevalence of *Candidatus* Neoehrlichia mikurensis in questing ticks at locations along the entire coastal strip of Norway from the Artic Circle in the North to the Swedish Border in the Southeast. *Ca*. N. mikurensis was detected by real time PCR targeting the *groEL* gene. *Ca*. N. mikurensis prevalence is high (> 6%) in the southeastern and northern parts of the coastal strip, but much lower (<1%) in locations along the west coast. This may be related to the mild and wet climate that characterizes this part of the country.

TBP-23
Prevalence and zoonotic potential of _Anaplasma phagocytophilum_ in roe deer from Spain

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Anaplasma phagocytophilum is a tick-borne bacterium with animal and public health significance. In Spain, *A. phagocytophilum* is mainly transmitted by *Ixodes ricinus*. Nevertheless, information on the animal reservoirs of this pathogen in this country is limited.
The aim of this study was to assess the prevalence and zoonotic potential of *A. phagocytophilum* in roe deer from Spain.
Spleen samples from 101 roe deer hunted in Spain during the 2015-2017 hunting seasons were collected. *Anaplasma* spp. DNA was firstly detected by a commercial qPCR (EXOone *Anaplasma* spp., EXOPOL, Zaragoza, Spain) and positive samples were characterized by sequence analysis of the *groESL* gene of *A. phagocytophilum*. Zoonotic potential of samples identified as *A. phagocytophilum* was assessed by comparing the obtained sequences with that reported as human pathogenic (GenBank accession no U96728).
Anaplasma spp. DNA was found in 64/101 spleen samples. Sequence analysis of *groESL* gene allowed the identification of *A. phagocytophilum* in 36 samples (35.6%). Single nucleotide polymorphisms (SNPs) allowed identifying 15 different *A. phagocytophilum* sequences, but none of them showed the same nucleotide sequence as the human pathogenic strain (98.2%-99.4% homology). In ten sequences, these SNPs implied 1-3 changes at the aminoacid level when compared to the human pathogenic strain; the remaining five sequences, corresponding to seven samples, showed the same aminoacid sequence than the zoonotic strain.

A. phagocytophilum is prevalent in roe deer from Spain, but our results suggest that most of the positive samples are not zoonotic. Since some isolates from roe deer showed the same aminoacid sequence than the human pathogenic strain at the *groESL* gene, further analyses should be conducted to achieve a more complete characterisation of *A. phagocytophilum* strains and thus to establish their zoonotic importance.
Supported by the Research Project 2016-CL018 (Asociación del Corzo Español).

TBP-25
First record of *Rickettsia vini* in *Ixodes lividus* ticks from sand martin (*Riparia riparia*) nests in Lithuania

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Introduction*: Ixodes lividus* (Koch, 1844) ticks are specific parasites of the sand martin *Riparia riparia* (L.). The distribution range of *I. lividus* covers Europe and Asia, and it can be found wherever there are sand martin nests. *Rickettsia* species are widespread in Europe and their distribution is dependent on the distribution of their reservoir or vector hosts. **Objectives**: In the present study, we report detection of rickettsial DNA in *I. lividus* ticks that were collected in Lithuania. **Materials & methods**: A total of 47 nests were collected in 3 different colonies of sand martin in the central part of Lithuania. In total, 2,770 ticks were found and identified as *I. lividus* based on morphological characteristics. The taxonomic identification of *I. lividus* was confirmed by sequence analysis of the tick mitochondrial 16S rRNA gene. All ticks were examined for the presence of rickettsial pathogens using PCR amplification of partial *gltA, ompA* and 17kDa antigen genes of *Rickettsia* spp. followed by DNA sequencing of positive samples for species identification.
Results: Phylogenetic analysis of 16S rRNA sequences revealed six genotypes of *I. lividus* and indicated that some genotypes were identical to those presented in western and central European populations, while others were unique for Lithuania. We detected DNA of *Rickettsia* in all stages of *I. lividus* ticks: in 90.6% of pooled larvae samples and in all examined 7 nymphs, 4 females and in one male specimen. Sequence analysis of partial *gltA, ompA* and 17kDa antigen genes showed 100% identity with the corresponding *Rickettsia vini* sequences obtained in GenBank.
Conclusion: This study represents the first record of *Rickettsia vini* in Lithuania and the importance of avian migratory connections, which are associated with the dispersal of *I. lividus* and rickettsial pathogens in northersn Europe.

TBP-27

Combination of microbiome analysis and serodiagnostics to assess the risk of pathogen transmission by ticks to humans and animals in central Germany

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Introduction: Arthropod-borne diseases remain a remarkable health-threat for humans and animals worldwide. To estimate the distribution of pathogenic agents and especially *Bartonella* spp., we conducted tick microbiome analysis and determination of the infection status of wild animals, pets and pet owners in the state of Hesse, Germany.
Methods: In total, 189 ticks collected from 163 animals were tested. Selected ticks were analyzed by next generation sequencing (NGS) and confirmatory PCRs, blood specimen of 48 wild animals were analyzed by PCR to confirm pathogen presence and sera of 54 dogs, one cat and 11 dog owners were analyzed by serology.
Results: Bartonella spp. were detected in 9.5% of all ticks and in the blood of 17 roe deer. Further data reveal the presence of the human and animal pathogenic genera *Spirochetaceae* (including *Borrelia miyamotoi* and *Borrelia garinii*), *Bartonella* spp. (mainly *Bartonella schoenbuchensis*), *Rickettsia helvetica*, *Francisella tularensis* and *Anaplasma phagocytophilum* in ticks. Co-infections with several genera were detected in nine ticks. One dog and five dog owners were seropositive for anti-*Bartonella henselae*-antibodies and one dog had antibodies against *Rickettsia conorii*.
Conclusions: This study shows the current image of pathogens circulating in ticks in central Germany. A broad range of tick-borne pathogens are present in ticks and animals, especially wild animals with possible implications for animal and human health. However, a low incidence of *Bartonella* spp. especially *Bartonella henselae* was detected. Ticks might serve as an excellent sentinel to detect and monitor circulating pathogens.

TBP-28

Microbiome analysis reveals the presence of *Bartonella* spp. and *Acinetobacter* spp. in deer keds (*Lipoptena cervi*)

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Introduction: The deer ked (*Lipoptena cervi*) is distributed in Europe, North America and Siberia and mainly infests cervids as roe deer, fallow deer and moose. From a one health perspective, deer keds occasionally bite other animals or humans and are a potential vectors for *Bartonella schoenbuchensis*.

This bacterium belongs to a lineage of ruminant-associated *Bartonella* spp. and is suspected to cause dermatitis and febrile diseases in humans.
Methods: In this study, we analyzed the microbiome from 130 deer keds collected from roe deer, fallow deer and humans in the federal states of Hesse, Baden-Wuerttemberg and Brandenburg, Germany and confirmed the results by conventional PCR methods.
Results: Endosymbiontic *Arsenophonus* spp. and *Bartonella* spp. represented the biggest portion (~90%) of the microbiome. Most *Bartonella* spp. (n=93) were confirmed to represent *B. schoenbuchensis*. Furthermore, *Acinetobacter* spp. were present in four samples, one of those was confirmed to represent *A. baumannii*.
Conclusions: These data suggest that deer keds harbor only a very narrow spectrum of bacteria which are potentially pathogenic for animals of humans.

TBP-30

Prevalence and molecular characterization of *Anaplasma phagocytophilum* in questing ticks from Galicia (NW Spain)

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Introduction: *Anaplasma phagocytophilum* is a zoonotic pathogen belonging to the family Anaplasmataceae. *Ixodes ricinus,* the most abundant tick in north-western (NW) Spain, is its main vector. Previous investigations performed in Spain reported the presence of *A. phagocytophilum* in questing *I. ricinus* with prevalences up to 20.5%; and human cases have also been reported. However, the number of studies using different genetic markers for characterizing *A. phagocytophilum* isolates is limited.
Objectives: The aim of this study was to determine the prevalence of *Anaplasma* spp. in questing ticks from NW Spain and the zoonotic potential of *A. phagocytophilum* isolates. The influence of several factors on the prevalence of this pathogen was also assessed.
Materials & methods: Ticks were monthly collected by flagging in three ecological regions (coast, plateau and mountain) during a two-year period. Overall, 1,056 *I. ricinus,* 12 *Ixodes frontalis,* 19 *Dermacentor marginatus* and 17 *Dermacentor reticulatus* were individually analysed. *Anaplasma* DNA was detected by a commercial qPCR assay (*Anaplasma* spp. EXOone, Exopol, Spain). Positive samples were confirmed and molecularly characterized at the gro*ESL, 16S rRNA* and *msp2* genes.
Results: *Anaplasma* DNA was found in seven *I. ricinus* (0.7%) and one *I. frontalis* (8.3%). Sequence analysis allowed the identification of non-human pathogenic strains of *A. phagocytophilum* in all samples. Using Chi-square test, no significant differences in the prevalence of *A. phagocytophilum* in *I. ricinus* were observed regarding the stage of development of ticks, ecological area and year of collection.
Conclusion: Our results reveal that the exophilic ticks from NW Spain showed low prevalences of non human-pathogenic *A. phagocytophilum,* and suggest that in the studied area the risk of acquiring this pathogen for human is very low although it should be considered.

TBP-31
Molecular detection of tick borne bacteria in questing *Ixodes ricinus* ticks in Northern Italy

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Introduction: *Ixodes ricinus* ticks are major competent vectors for a range of microbial pathogens, several of which are of medical importance.The occurrence of bacteria such as *Rickettsia* spp., *Borrelia* spp. and *Anaplasma phagocytophilum* in questing ticks is yet a relevant question.
Objectives: Our objectives were to identify the prevalence of *Rickettsia* spp., *Borrelia* spp. and *Anaplasma phagocytophilum* in questing ticks in the Province of Trento, establish co-infection rates, and compare infection levels of selected pathogens.
Materials & methods: In 2011-2013 ticks were collected by dragging. All the ticks were identified, DNA was extracted and a nested PCR was carried out for the analysis of *Rickettsia* spp., *Borrelia* spp. and *Anaplasma phagocytophilum*. A PCR targeting the ompA gene was performed to detect the presence of *Rickettsia monacensis*. All positive samples were sequenced and a Blast search was performed.
Results: We collected and analyzed 2,133 questing ticks (1,794 nymphs and 339 adults) belonging to *Ixodes ricinus*. Nine tick-borne bacteria were detected: *B. garinii* (7.2%), *Rickettsia helvetica* (5.6%), *B. afzelii* (4.6%), *B. valaisiana* (4.6%), *Borrelia burgdorferi* s.s. (4.3%), *R. monacensis* (3.5%), *Anaplasma phagocytophilum* (1.9%), *B. lusitaniae* (0.8%) and *R. slovaca* (0.04%). 32% of the ticks resulted infected with at least one pathogen and 2.1% with two.
Conclusion: The high percentage of ticks found positive to at least one of the bacteria detected poses serious concerns regarding the potential of transmitting pathogens to humans. Carriage of multiple pathogens was observed, demonstrating the risk of acquiring multiple infections as a consequence of a tick bite.

TBP-32
Tick-transmitted diseases in horses in central Germany: Clinical signs, clinicopathological findings, diagnosis, treatment and outcome

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Introduction: Tick-transmitted causative agents in horses which were described in Germany are tick-borne encephalitis virus, *Anaplasma phagocytophilum* (formerly *Ehrlichia equi*), *Borrelia burgdorferi*, *Babesia caballi* and *Theileria equi*.
Objectives: The aim of this study was to evaluate the most important clinical signs, hematological and serological findings, treatment and outcome of clinical cases with tick-borne diseases.
Material & methods: A retrospective study of cases with anaplasmosis or piroplasmosis were included. Most important clinical signs and blood examination findings were described. Seroprevalence were done with IFAT. The treatment of horses with anaplasmosis was done with fluids and 7 mg oxytetracycline/kg bw/d i.v. over 7 days. The horse with piroplasmosis was treated continously

with fluids and partial nutrition including 4 mg imidocarb dipropionate /kg bw i.m.
Results: In the last six years seven horses had anaplasmosis. Signs: moderate depression, fever 41° C, petechiae in the mucous membranes, edema, thrombocytopenia (18-90 G/L, normal range 105-330), 6/7 with ticks on the skin, 1/7 horse without ticks diseased in December, *A. phagocytophilum* inclusions in neutrophils of all patients, IFAT and PCR were positive. All horses were discarged. The seroprevalence tested with IFAT was between 29 and 33%. Only one horse with *Babesia caballi* merozoites in erythrocytes, icterus, edema, thrombocytopenia (58 G/L), positive IFAT and PCR had acute renal failure and died. Seroprevalence was 6,1% for *Theileria equi*.
Conclusion: Based on these clincal findings and seroprevalence results tick-borne diseases anaplasmosis and piroplasmosis are in Germany!

TBP-33
Vector-borne parasites in Namibian cheetahs (*Acinonyx jubatus*) and leopards (*Panthera pardus*)

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Introduction: The numbers of free-ranging cheetahs (*Acinonyx jubatus*) and leopards (*Panthera pardus*) are rapidly declining which stresses the importance of investigating their health status and infection risk. Free-ranging cheetahs and leopards do not show signs of infectious diseases, although they are regularly exposed to directly-transmitted and foodborne pathogens. Information on vector-borne pathogens, however, remains scarce.
Objectives: This study aimed at determining the prevalence of hemoparasites in free-ranging cheetahs and leopards from Namibia, with a focus on co-infections in host animals.
Material & methods: Blood samples collected from 52 cheetahs and 57 leopards were screened for helminths (Onchocercidae), apicomplexa (*Babesia*, *Theileria*, *Hepatozoon*) and bacteria (Anaplasmataceae, *Rickettsia*) by PCRs targeting the ITS-2, 18S, 16S rRNA and *glt*A genes, respectively.
Results: Cheetahs were PCR positive for all examined hemoparasites, with 63.5% of cheetahs being positive for Onchocercidae (all *Acanthocheilonema*), 59.6% for *Babesia*, 78.9% for *Hepatozoon*, 71.2% for *Anaplasma* and 15.4% for *Rickettsia*. Leopards were PCR negative for *Rickettsia* and had lower prevalence for the other hemoparasites than cheetahs: 35.1% were positive for *Acanthocheilonema*, 56.1% for *Babesia*, 66.7% for *Hepatozoon* and 43.9% for *Anaplasma*. Cheetahs were significantly more often co-infected (50%) with helminths, bacteria and apicomplexan parasites than leopards (10.5%) ($p<0.001$). None of the animals showed clinical signs of infectious diseases.
Conclusion: Our results suggest a higher exposure and/or transmission of hemoparasites in cheetahs than in sympatric leopards. Although cheetahs had high prevalence of co-infections, they were clinically healthy. This suggests that free-ranging Namibian cheetahs respond effectively to challenges induced by parasites. This is the first study that compared hemoparasites in sympatric, free-ranging cheetahs and leopards, and represents the groundwork for future studies of host-parasite interaction in these threatened species.

TBP-34
Screening of Ticks Collected in Sudan for Diverse Viruses

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Introduction: The global number of detected tick-borne virus infections is increasing, and several novel and divergent tick-associated viruses have been documented recently. Sudan hosts a variety of tick species known to transmit viruses that are pathogenic to humans. Crimean-Congo haemorrhagic fever virus (CCHFV) is endemic in Sudan and causes sporadic outbreaks of disease in human populations. Despite this, knowledge regarding the prevalence of CCHFV and other tick-borne viruses in Sudan remains fragmentary.
Objectives: Objective 1: investigation of the prevalence and distribution of tick-borne viruses across Sudan, in order to assess the risk for transmission to human populations.
Objective 2: phylo-geographical investigation of ticks across Sudan.
Materials & methods: Ticks were collected from domesticated animals and the environment in 8 states in Sudan. Ticks were assayed for the presence of viruses using a pan-phlebovirus PCR and a specific CCHFV PCR. In addition to morphological identification, PCR assays targeting the COX, ITS2, 16S rRNA and 12S rRNA were performed to gain molecular information about the tick species.
Results: Preliminary results revealed that 37 % of tested tick pools from different states were positive when assayed with the pan-phlebovirus PCR assay. One tick pool was positive for CCHFV. The molecular tick identification is in agreement with the morphological identification.
Conclusion: Preliminary results of the pan-phlebovirus assay indicate that multiple tick-associated viruses are present in widespread areas of Sudan, although the risk to human populations from these tick-associated viruses remains to be determined. The detection of CCHFV in a tick pool from Eastern Sudan confirms for the first time the circulation of CCHFV in ticks in this region. Species identification by both morphological and molecular methods enables phylo-geographical investigations and sheds light on the possible movement of these ticks and the viruses they transmit.

TBP-36
Population genetic analysis and sub-structuring of *Theileria annulata* in Sudan

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Introduction: Tropical theileriosis, cause by *Theileria annulata*, is a major obstacle for improvement of cattle production in Sudan. The disease is endemic in the north and central parts of Sudan. Due

to changes in the tick vector distribution, outbreaks of the disease have been reported outside the known endemic areas, in east and west parts of the country.
Objectives: To control the disease, a live attenuated vaccine has been developed. Before the vaccine is deployed there is need to genotype the parasite in the field and compare it with the vaccine strain.
Materials & methods: A total of 246 field isolates and the vaccine strain were genotyped using nine microsatellite markers.
Results: High multiplicity of infection was reported from north and central populations compared to east and west populations. Linkage analysis indicated that only the north population was in linkage disequilibrium, while other populations were in linkage equilibrium, and when all isolates were treated as single population linkage disequilibrium was indicated. Nei genetic identity between the vaccine and field isolates ranged between 0.62 with north and 0.39 with west population.
Conclusion: These results suggest that the vaccine may be suitable for deployment in all areas where tropical theileriosis occurs.

TBP-37
Alkhurma hemorrhagic fever virus RNA in *Hyalomma* ticks infesting migratory birds

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Alkhurma hemorrhagic fever virus (AHFV) was identified in 1995 after an outbreak of viral hemorrhagic fever in the Jeddah Province in Saudi Arabia. The clinical manifestation of Alkhurma hemorrhagic fever (AHF) includes malaise and influenza-like symptoms followed by encephalitis and ecchymosis. The case fatality is reported to be as high as 25% but could be lower since mild cases probably are undiagnosed. An African origin has been suggested for AHFV, as well as ticks being potential vectors and goats, sheep, and camels potential reservoirs. AHF is endemic in several western provinces of Saudi Arabia and the case frequency is increasing in the region. Clinical cases have been reported in Africa near the Egypt-Sudan border and seropositive patient and ticks with AHFV RNA have been detected in Djibouti, indicating a possible wider geographical area for the virus. In the light of these findings, we investigated whether ticks infesting migratory birds en route from Africa to Europe and Asia during spring migration carry AHFV. Ticks were collected from northward migratory birds caught at eight bird observatories in the Mediterranean basin during the spring of 2009, 2010, 2014, and 2015 and screened for AHFV by qPCR. In total 36,893 birds were caught and 1,771 ticks collected. AHFV RNA was detected in six (n=6) *Hyalomma marginatum* sensu lato ticks (H. rufipes) collected from two Sedge warblers (*Acrocephalus schoenobaenus*), one Eastern woodchat shrike (*Lanius senator niloticus*), and one Western yellow wagtail (*Motacilla flava*) caught in Greece and one Common redstart (*Phoenicurus phoenicurus*) caught in Turkey, raising concerns for dissemination of the virus to novel areas.
Increased surveillance and further investigations of the ecology and the modes of transmission of AHFV, including the role of H. marginatum s.l. ticks as vectors and passerine birds as reservoirs or distributors of potentially infected ticks, are needed.

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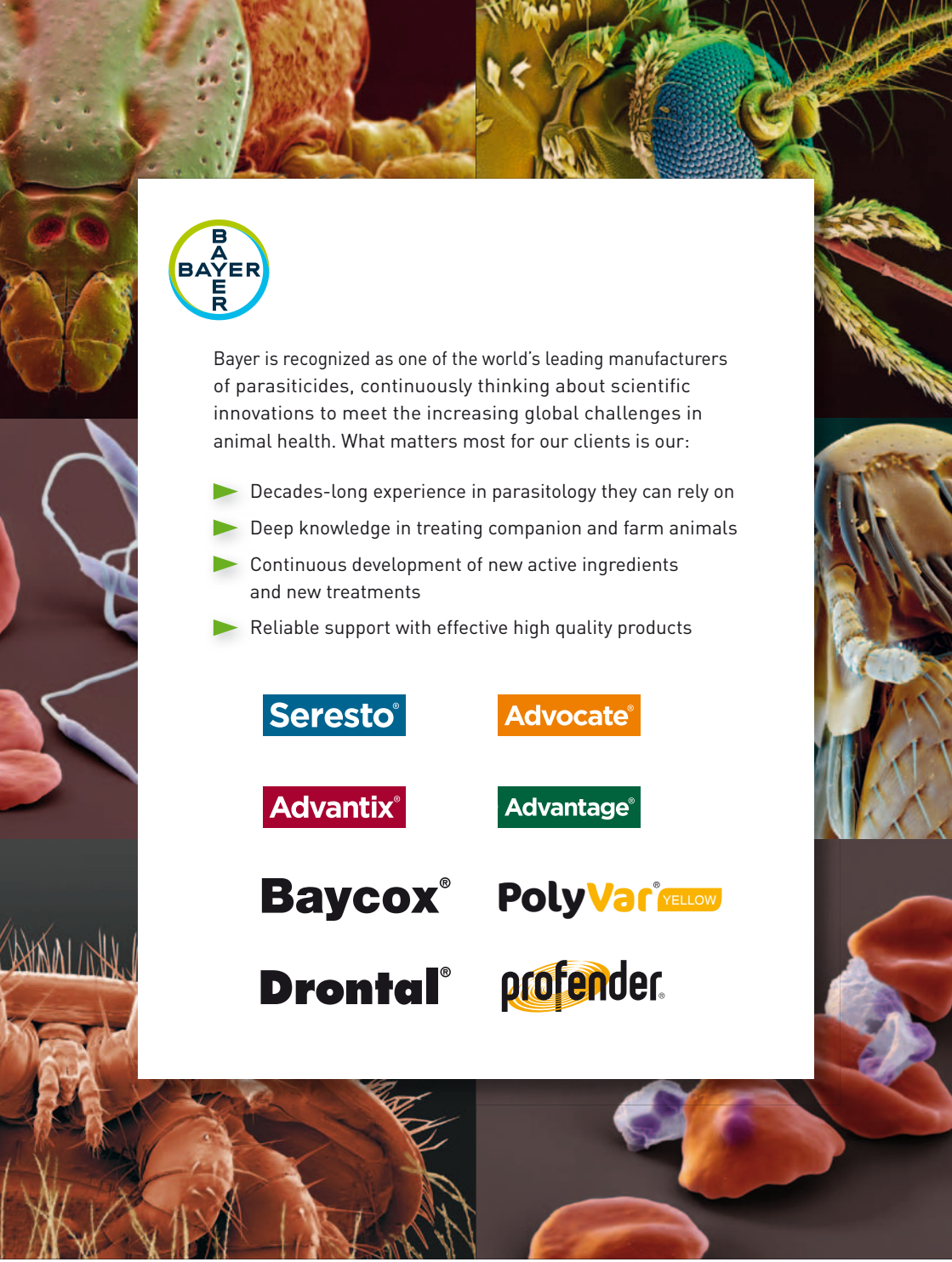

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Quelle Illustration: Pfizer

*Frühsommer-Meningoenzephalitis

	FSME	Lyme-Borreliose
Erreger	FSME-Virus	Bakterium (Borrelia burgdorferi)
Erregerlokalisation	Speicheldrüsen	Mitteldarm
Übertragungszeit	Sofort nach dem Stich	Ca. 12–24 Std. nach dem Stich
Therapie	Keine ursächliche	Antibiotika
Impfung	Ja	Nein

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