# International Symposium on Tick-Borne Pathogens and Disease

## ITPD 2019 Vienna, Austria
## 8 to 11 September 2019

Under the auspices of the Austrian Society for Hygiene, Microbiology and Preventive Medicine (ÖGHMP)

**Organisers**

ÖGHMP and ESGBOR

**Venue**

Parkhotel Schönbrunn, Vienna, Austria

# BOOK OF ABSTRACTS

ÖGHMP
Österreichische Gesellschaft für Hygiene, Mikrobiologie und Präventivmedizin

**ÖGHMP**
Austrian Society for Hygiene, Microbiology and Preventive Medicine

and

ESCMID

**ESGBOR**
ESCMID Study Group for Lyme Borreliosis

and

INSTAND

**Instand e.V.**
Society for promoting
Quality Assurance in Medical Laboratories e.V.

# Abstracts
# of presentations
# during the plenary sessions

A1 **Ticks in Germany – results of recent flagging studies**

Olaf Kahl[a], Ingrid Bulling[a], Daniel Kämmer[a], Lidia Chitimia-Dobler[b,c], Franz Rubel[d]

[a] tick-radar GmbH, Berlin, Germany
[b] Bundeswehr Institute of Microbiology, Munich, Germany
[c] Department of Parasitology, Institute of Zoology, University of Hohenheim, Stuttgart, Germany
[d] Unit for Veterinary Public Health and Epidemiology, University of Veterinary Medicine, Vienna, Austria

The dominating hard tick species in central Europe is *Ixodes ricinus*, a principal vector of various pathogens, e.g. *Borrelia burgdorferi* sensu lato and tick-borne encephalitis virus. Another rather common tick species in parts of Germany is *Dermacentor reticulatus*, a vector of TBE virus, *Babesia canis*, *Rickettsia raoultii*, and *Rickettsia slovaca*. A few other exophilic tick species occur more or less sporadically in Germany. For example, recent research identified *Ixodes inopinatus*.

There has been an ongoing project with monthly flagging excursions at 23 locations in Germany beginning in February 2018 (Project A) and a further flagging project with only one visit to each of 29 locations from April to June 2018 (Project B), both supported by Pfizer Deutschland GmbH. All nymphal and adult ticks and some larvae were morphologically determined down to species.

Although *I. ricinus* dominated in all our collection sites, *I. inopinatus* was found at each of 23 locations in Project A and at 21 of 29 locations in Project B. The density of questing *I. inopinatus* nymphs and adults in 2018 was approximately 9% of that of *I. ricinus*. The rarely found bird tick *Ixodes frontalis* was collected at 8 locations in 5 German states, altogether 13 larvae, 6 nymphs, and 2 adults so far. All the *Ixodes frontalis* larvae were collected in the cold season even at temperatures down to 0°C (November to March). Updated distribution maps will be shown from various exophilic tick species found in Germany.

Not all flagged *Ixodes* ticks in Germany are *I. ricinus*. *Ixodes inopinatus* appears to be widespread in Germany, and *I. frontalis* might often be overlooked in flagging studies in Europe.

A2 **Increase of tick density over a 15-year period on Chaumont Mountain in Switzerland**

Cindy Bregnard 1, Olivier Rais 2 & Lise Gern, Maarten J. Voordouw 1, 3

1 Laboratory of Ecology and Evolution of Parasites, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland,
2 Laboratory of Ecology and Epidemiology of Parasites, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland
3 Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada

Climate change in Europe is believed to be driving the expansion of *Ixodes ricinus* ticks towards northern latitudes in Scandinavia and towards higher altitudes in Central Europe. Abiotic factors such as temperature, relative humidity, and saturation deficit influence tick questing behavior and life history traits, and ultimately tick phenology and abundance. Given the importance of alpine tourism and leisure activities in Switzerland, changes in the altitudinal abundance of *I. ricinus* ticks may have important consequences for the risk of exposure to tick-borne pathogens. To determine how climate and elevation might affect the abundance of *I. ricinus* ticks, we used data from a long-term study that had monitored the abundance of nymphal and adult *I. ricinus* ticks at four different elevations on Chaumont Mountain in Neuchatel, Switzerland. These four elevation sites were sampled on a monthly basis over a period of 15 years from January 2004 to December 2018. We used AIC-based model selection to test whether yearly mean climate variables such as temperature, relative humidity, and saturation deficit could explain annual variation in tick density. Variation in nymph density was best explained by elevation and year, whereas variation in adult density was best explained by nymph density in the previous year. Nymph density increased over the study period at the three lower elevations and the increase was significant for the lowest elevation, but adult density remained stable over the study period. The yearly mean climate variables failed to explain the annual variation in tick density on Chaumont Mountain. Our 15-year study shows that finding a signal of climate change on tick density in endemic areas is difficult.

A3 **Migratory birds, their ticks and pathogens – a new challenge?**

Georg Gerhard Duscher

Institute of Parasitology, University of Veterinary Medicine, Vienna, Austria

Every year in spring time, migratory birds carry larvae and nymphs of foreign tick species e.g. *Hyalomma* spp., along their way through Europe. During the bird´s journey the ticks drop off and in case of suitable prevailing weather conditions, those ticks are able to develop further to adult ticks and quest for new hosts in the new habitat. So happened in October last year in the north of Austria, where an adult male *Hyalomma marginatum* was found on a Haflinger horse. Additionally this tick was harbouring *Rickettsia aeschlimannii*, clearly pointing out the risk of importation of "exotic" diseases such as Crimean-Congo Hemorrhagic Fever virus (CCHFv), which can be transmitted by especially this tick species. This phenomenon was observed in several European countries, with up to 50% of *Rickettsia* spp. positive ticks.

During a study at a bird ringing station in Italy, migratory birds were found harbouring mainly *Hyalomma* spp. ticks. Out of these 20.1 % were tested positive for "spotted-fever-group" rickettsiae comprising *Rickettsia aeschlimannii*, *Rickettsia africae* and *Rickettsia raoultii*. None of the ticks were found positive for CCHFv.

Migratory birds constantly introduce foreign tick species to northern areas. With these tick species new exotic pathogens such as *Rickettsia aeschlimannii* are transported. This has to be considered in terms of human and veterinary medicine. The public should be trained to notice "new and exotic" tick species. Even more, there is a possibility that those ticks are able to overwinter and consequently are on the edge of developing a reproductive population in northern areas.

A4 **Environmental and behavioral impacts on tick infestation of a Borrelia-amplifying, avian host**

Robert E. Rollins a, Alexia Mouchet b, Gabrielle Margos c, Volker Fingerle c, Noémie Becker a, Niels Dingemanse b

a Evolutionary Biology Group, Ludwig-Maximilians University, Munich, Germany; b Behavioural Ecology Group, Ludwig-Maximilians University, Munich, Germany; c National Reference Center for Borrelia, Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim, Germany

Many vertebrates, including multiple bird species, are implicated as tick host and act as reservoirs for tick borne pathogens, such as *Borrelia burgdorferi* sensu lato. *B. burgdorferi* s. l. refers to a group of bacterial species, some of which are the agents of Lyme borreliosis in humans. Research on the ecological drivers of tick load is incomplete in the European system, especially in relation to animal behavior and milder forms of human disturbance (i.e. human presence). Our study aims to understand avian tick infestation in a model host species, the cavity-breeding great tit (*Parus major*), utilizing 12 nest-box plots in southern Germany taking bird behavior and human disturbance into account. We expect bird behavior and human disturbance to impact host, tick, and pathogen relationships as shown in the North American system. Great tit pairs were monitored during the breeding season (2017-2018) for general life history traits, tick load, and exploratory behavior. In parallel, human disturbance was quantified as the number of recreants observed in a plot. We investigated spatiotemporal variation in tick infestation (probability of tick infestation, tick load given being infested), and whether it varied as a function of the behavioral phenotype of the bird and in relation to human disturbance. Plots varied in their proportion of infested birds and average tick load. Infestation probability decreased with human disturbance, but bird behavior did not impact tick infestation contrary to our predictions. Ticks were collected from birds in 2018 and screened for *B. burgdorferi* s. l.. All plots had *Borrelia*-positive ticks with five genospecies being identified; four of which are human pathogens. The probability of a tick to be *Borrelia* positive was heavily impacted by bird identity lending support to the presence of systemically infected hosts within our test plots.

A5 **What is the Significance of Candidatus Borrelia kalaharica in the cause of febrile illness in Nigeria?**

Adefolake Bankole, Nusirat Elulu and Sally Cutler

University of East London, United Kingdom

Tick-borne relapsing fever (TBRF) is under reported/underdiagnosed in Nigeria, even though patients present with similar symptoms to TBRF. A recent pilot study in Nigeria reported the presence of *Candidatus* Borrelia kalaharica in 6% of soft ticks screened. To further explore the tick vector and determine its impact on humans, more samples were obtained from tick endemic regions including Plateau and Borno States in Nigeria. A total of 100 soft and hard ticks were picked around farmlands and market places in Borno and Plateau states. A total of 319 animal sera such as cattle, sheep, goats and dogs were obtained from Barkin Ladi (09.31334°N, 08.49014°E; 09.26292°N, 08.56289°E), Bokkos (09.19024°N, 08.55197°E) and Toro (10.08679°N, 08.59766°E) local government areas of Plateau state. While a total of 148 cattle sera was obtained from Jere (N 11.84692°, 13.15712°E) local government in Borno state. 152 finger prick samples and Giemsa stained blood smear were collected on FTA cards and glass slides respectively from febrile patients in the two states. 102 finger prick samples from Plateau state teaching hospital and Jos University teaching hospital, and 50 samples from Borno state specialist hospital. DNA was extracted from ticks and blood samples. Ticks were identified using conventional PCR targeting their 16S rRNA genes, amplicons were cleaned, followed by Sanger sequencing. Ticks were also identified morphologically using entomological keys previously described. DNA was screened for *Borrelia* and other tick-borne pathogens including *Rickettsia*, *Babesia* spp. via RT-PCR. Reactive samples were confirmed via conventional PCR and sequencing. Spirochetes were determined microscopically in Giemsa stained blood smears. *Borrelia* was further characterized by sequencing several loci of 16s RNA, *glpQ*, *flaB* and published Multilocus Sequence Analysis of housekeeping genes.

Note: Analysis is ongoing and results will be presented at the conference.

## A6 **Tick-borne bacterial infections in Austria - outcomes of tick bites analysed in a prospective study**

Mateusz Markowicz, Anna Margarita Schötta, Dieter Höss, Hannes Stockinger, Gerold Stanek

Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna

**Objectives** The aim of this study was to investigate the outcomes of tick bites in adults bitten 2014-2018. The analysis focused on tick-borne bacteria including *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia* sp., *Candidatus* neoehrlichia mikurensis and relapsing fever borreliae.

**Patients and Methods** Information about the site of the tick bite, the duration of the tick attachment and the origin of the tick was collected. Serological testing and PCR from blood was performed for the pathogens in the first week after tick bite and approximately 6 weeks later. The endpoint of the study was defined as at least one of the following: 1. occurence of erythema migrans (EM); 2. increase of the antibody level in the follow-up sample; 3. presence of the microorganism determined by PCR in the blood in the initial or the follow-up sample. In total, 489 persons (259 female and 230 male) bitten by 1295 ticks were included in the study. Seven participants were lost for follow-up.

**Results** *Borrelia* infection was found in 25 (5%) participants. In one of them the infection occurred twice. In this group, 15 patients had EM. Eleven (2%) patients were tested positive for *C.* neoehrlichia mikurensis by real-time PCR. Three of them were positive in the first and the follow-up examination. None of these patients had fever or any other symptoms. Finally, there was one asymptomatic patient infected with *Borrelia miyamotoi* determined by PCR.

**Conclusions** Our study confirms previous findings that transmission of Lyme *Borrelia* is the most frequently detected bacterial tick-borne pathogens. In our cohort, 5 % of the participants became infected with this pathogen. Surprisingly, infection with *C.* neoehrlichia mikurensis was the second most common infection; we can demonstrate that tick bitten persons may carry this pathogen for several weeks which was not observed for any other pathogens tested in the study.

A7 **Remote sensing of innate killers**

Alexander Platonov, Vladimir Dubyansky, Anton Titkov, Ivan Uvarov, Vladimir Tolpin, Nadezhda Kolyasnikova, Daria Prislegina, Stefan Raichich and Victor Maleev

Central Research Institute of Epidemiology, Moscow, Russia; Space Research Institute of the Russian Academy of Sciences, Moscow; Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Sciences, Moscow, Russia

In Russia most important tick-borne diseases (TBD) are tick-borne encephalitis, *Ixodes* tick-borne borrelioses, Crimean-Congo hemorrhagic fever, Astrakhan rickettsial fever, and Siberian rickettsial fever. At least 10,000 cases of these TBD with 50 deaths have been officially registered annually; economic losses caused by TBD exceed 2 billion rubles per year. The nature of epidemic process of TBD assumes its dependence on the weather conditions and the current state of ecosystems in short terms, and on long-term climate changes and ecosystem evolution. From technological standpoint the most informative and reliable source of data on environmental indicators in the vast territory of Russia are the data of the Earth remote sensing from space. The capabilities of the Space Research Institute allow both to conduct daily real-time monitoring of various ecosystems in Northern Eurasia and work with nearly 20-year old archives of satellite data with excellent temporal and spatial resolution. On the other hand, Central Research Institute of Epidemiology possesses the data on the TBD incidence for this period stratified by region, time interval, population groups, and so on. The objective of present study is to combine the environmental and epidemiological data sources and analyze those using adequate statistical methods (correlation and regression analysis, building of “decision trees”, cluster analysis, etc.) and mathematical modelling. The “explanatory” models account for the values of weather and climatic parameters both during “epidemic period” (i.e. in the season when 90-95% cases of certain TBD incidence are registered) and in the year or years preceding the “epidemic period”. The “prognostic” models assume using the values of environmental indices only before the start of the “epidemic period”, i.e. they are intended for real forecast of TBD incidence and shall be used also for practical purposes. This study is supported by the Russian Science Foundation (project 19-75-20088).

A8 **Human Babesiosis: molecular characterization of a new etiological agent in the "B. divergens species complex"**

Claire Bonsergent, Nathalie de la Cotte, Maggy Jouglin, Marie-Charlotte de Carné, V. Perronne, L. Malandrin

BIOEPAR, INRA, Oniris, 40307 Nantes, France, Service de Maladies Infectieuses et Tropicales, Hôpital F. Quesnay, 78200 Mantes-la-Jolie, France

Human babesiosis is a tick-borne infectious disease caused by the multiplication of protozoa of the genus *Babesia* in erythrocytes. Severe and often fatal human babesiosis in Europe occurs in immunocompromised patients and are mainly due to *B. divergens*. A 56 year-old man, splenectomised in 2001, came into the emergency room for meningitidis syndrom suspicion on September 2017. Patient suffered fever and headaches for 2 weeks. Then appeared important asthenia, sweats, fast breathing, myalgia, and arthralgia. Babesiosis was diagnosed on blood smear, confirmed by serology and PCR. The patient was successfully treated.

As symptoms were atypically mild for a *B. divergens* infection (usually fulminant and often fatal), we performed a molecular characterisation of the etiological agent based on several conserved (18S rDNA) or variable molecular markers (*ama-1* - apical membrane antigen-1 - and *rap-1* – rhoptry associated protein-1). We performed the same analysis on *B. divergens*, as well as on the phylogenetic related deer pathogen *B. capreoli* and the american zoonotic *Babesia* sp. MO-1.

The 18S rDNA sequence analysis indicated that this parasite differs from *B. divergens*, *B. capreoli* and *Babesia* sp. MO-1, but is more closely related to the american zoonotic *Babesia* sp. MO-1. The analysis of the other molecular markers (*ama-1* and *rap-1*) on several strains of *B. divergens*, *B. capreoli* and *Babesia* MO-1 demonstrated a low genetic diversity intra-species and a greater diversity between different species. Sequences of the new parasite differed from all of them.

The patient was infected by a tick-bite on a French island. This strain has probably evolved separately from the mainland *Babesia* species. As *B. divergens*, *B. capreoli* and *Babesia* sp. MO-1 have different natural hosts (cattle, roe deer and cottontail rabbits respectively), this new isolate may also have a specific natural host. Its proposition as a new species is debated.

A9 **Ticks are rich sources of anti-haemostatics**

Maria Kazimirova

Institute of Zoology, Slovak Academy of Sciences

Ticks are obligate blood feeders. During the long-lasting co-evolution with their vertebrate hosts, ticks have developed powerful mechanisms to counteract different arms of the hosts` defence systems (haemostasis, immune responses, wound healing) and ensure successful feeding and survival. Haemostasis is the first line of defence against the tick bite and comprises a series of physiological processes that stop bleeding at the site of vascular injury by formation of a haemostatic plug. Three major mechanisms are involved in haemostasis: vasoconstriction (termination of bleeding from damaged blood vessels), coagulation (production of a fibrin clot), and formation of a platelet plug. The enzymes in the coagulation cascade are activated through different pathways, depending on various endogenous and exogenous factors. Tick saliva, injected into the feeding lesion in the host skin is the main source of the anti-haemostatic compounds such as vasodilators, inhibitors of platelet aggregation, and anticoagulants targeting different factors of the blood coagulation cascade. Saliva of a single tick species contains a wide variety of molecules of which some may target single coagulation factors whereas others can have multiple functions. A number of inhibitors of serine proteases involved in the coagulation cascade have been identified and characterised from ticks. Thrombin and factor Xa are the most common targets for majority of the identified tick anticoagulants. Compounds derived from tick saliva represent potentially useful therapeutic agents for treatment of haemostatic disorders. In spite of the wide range of the identified inhibitors derived from tick salivary glands, only a limited number passed pre-clinical and clinical tests. However, the specific binding mode of tick anticoagulants to their target molecules makes them important molecular tools to increase our understanding of the mechanisms of host blood coagulation. Information on the structure and function of tick-derived anticoagulants can also be used in designing synthetic peptides as a basis for development of novel drugs.

## A10 **Artificial feeding of ticks**

Michiel Wijnveld

Institute for Hygiene and Applied Immunology of the Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria.

It always is difficult to study ticks under laboratory conditions due to their need of a blood meal several times during their lifespan. Over the years, alternatives to the direct use of laboratory animals were developed and are continuously optimised.

Several systems have been invented or adapted for feeding ticks artificially. All artificial systems must include either a natural membrane (e.g. mammalian skin) or an artificial membrane (e.g. silicon or parafilm based). All setups provide the ticks with fresh blood to feed upon, a system to keep the blood at a certain temperature and a way to provide humidity.

Importantly, the membrane used, needs to be sufficiently thick to support the ticks but thin enough for the ticks to pierce through and reach the blood. Moreover, the blood anticoagulants must be chosen depending on the assay and tick species used. Defibrinated blood is most often used and the blood donor should be chosen accordingly as well; in particular for soft ticks, as the ticks might die after feeding upon blood from the wrong host.

Hard tick artificial feeding takes place over several days. Depending on the species and life stage, feeding can take several days to weeks before feeding is completed. In contrast, soft tick artificial feeding time can be anywhere within several minutes to hours, making the procedure much faster and less complex.

Difficulties experienced during artificial feeding are primarily related to unwillingness of ticks to bite. For example, the membranes might not be attractive enough or other stimuli are absent. Also, ticks might not be ready to feed especially shortly after moulting. Another problem is caused by the contamination of the blood, leading to detaching, or death of the ticks.

A11 **Transmission of Rickettsia raoultii and Rickettsia massiliae DNA by Dermacentor reticulatus and Rhipicephalus sanguineus s.l. ticks during artificial feeding**

Emanuela Olivieri (a), Michiel Wijnveld (b), Marise Bonga (c), Laura Berger (c), Fabrizia Veronesi (d), Maria T. Manfredi (e), Frans Jongejan (c)

(a) Dipartimento di Biologia e Biotecnologie, Università di Pavia, Italy, (b) Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, (c) Utrecht Centre for Tick-borne Diseases (UCTD), FAO Reference Centre for Ticks and Tick-borne Diseases, Faculty of Veterinary Medicine, Utrecht University, The Netherlands, (d) Department of Veterinary Medicine, University of Perugia, (e) Department of Veterinary Medicine, Università degli Studi di Milano

Spotted fever group (SFG) rickettsiae are recognised agents of emerging infectious diseases in humans and animals. A plethora of SFG *Rickettsia* species has been identified in ticks recovered from human and animal patients. However, the detection of rickettsial DNA in ticks does not necessarily mean that these ticks can act as vectors for these pathogens. Here, we used artificial feeding of ticks to get a better understanding of the transmission dynamic of *Rickettsia massiliae* and *Rickettsia raoultii* by *Rhipicephalus sanguineus* (sensu lato) and *Dermacentor reticulatus* ticks, respectively.

An artificial feeding system based on silicone membranes were used to feed adult *R. sanguineus* (s.l.) and *D. reticulatus* ticks. Blood samples from in vitro feeding units were collected at regular intervals and analysed for the presence of rickettsial DNA using PCR and reverse line blot hybridisation.

The attachment rate of *R. sanguineus* (s.l.) ticks were 40.4% at 8 h post-application, increasing to 70. 2% at 72 h. *Rickettsia massiliae* was detected in blood samples collected 8 h after the *R. sanguineus* (s.l.) ticks were placed into the in vitro feeding units. *D. reticulatus* ticks were pre-fed on sheep and subsequently transferred to the in vitro feeding system. The attachment rate was 29.1 % at 24 h post-application, increasing to 43.6 % at 96 h. *Rickettsia raoultii* was detected in blood collected 24 h after *D. reticulatus* was placed into the feeding units.

*Rhipicephalus sanguineus* (s.l.) and *D. reticulatus* ticks are vectors of *R. massiliae* and *R. raoultii*, respectively. The early transmission time of *R. massiliae* after tick attachment emphasises the importance of removing ticks as soon as possible to minimise transmission. This study highlights the relevance of in vitro feeding systems to provide insight into the vectorial capacity of ticks and the dynamics of tick-borne pathogen transmission.

A12 **Tick attachment cement as biomimetic template for a potential tissue glue**

Johannes Suppan1; Benedikt Engel2; Martina Marchetti-Deschmann2, 4, O. Andriotis5, P. Thurner4, 5, Laura Maers6, Markus Valtiner6, Sylvia Nürnberger1, 3, 4

1 Medical University of Vienna, Department of Orthopedics and Trauma-Surgery, Division of Trauma-Surgery, Vienna, Austria, Email: sylvia.nuernberger@meduniwien.ac.at 2 TU Wien, Institute of Chemical Technologies and Analytics, Vienna, Austria, 3 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria 4 Austrian Cluster for Tissue Regeneration, Vienna, Austria, 5 TU Wien, Institute of Lightweight design and structural biomechanics, Vienna, Austria, 6 TU Wien, Institute for Applied Physics, Vienna, Austria

Many ixodid ticks secrete a sticky material, the “cement", while they anchor to a host with their mouthparts [1]. Due to its potential to bond to humid and even wet tissue, the cement is of interest for medical glue research, aiming to replace toxic tissue adhesives, screws and metal plates.

In the current study we investigated the cement of *Dermacentor marginatus*, harvested from artificial feeding units and analysed its morphological, physical, and bioanalytical properties. Morphological methods included high-resolution stereo microscopy, various histochemical staining and electron microscopy (SEM, TEM). Atomic force microscopy (AFM) and surface force apparatus (SFA) were used to assess the mechanical and adhesive properties. Biochemical analyses comprise amino acid quantification by gas chromatography electron ionization tandem mass spectrometry (GC-EI-MS/MS) and protein identification by 1D electrophoresis and liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).

The overall structure of the cement revealed a droplet-like sub-compartmentation and partial positive staining for lipids with oil red. Histochemically, the most dominant staining gave Biebrich Scarlet for basic proteins. Moderate PAS-positive regions indicated carbohydrates at the margins of the cement cones and Alcian blue staining was almost completely absent. Arnow staining for DOPA, a typical compound of several other bio-adhesives, was negative. TEM revealed locally different regions and particular internal ultrastructural features. AFM indicated viscoelastic properties and the pull-off tests the adhesiveness of the material. Amino acid quantification confirmed the absence of DOPA, and found glycine as the most abundant amino acid followed by leucine and serine. Protein analysis gave hints for proteins found in ticks but also in other adhesive species.

The current findings indicate that the tick cement is an interesting candidate for a biomimetic glue template since it differs in many ways from previously identified biological glues and suggests a yet not known adhesive mechanism.

1 Suppan et.al. *Biol.Rev.Cambr.Phil* **93**(2)1056 (2017)

A13 **Association of Statin Use and Microbiological and Clinical Characteristics of Early Lyme Borreliosis**

Maša Velušček 1, Rok Blagus 2, Tjaša Cerar 3, Eva Ružić Sabljić 3, Franc Strle 1, Daša Stupica 1,

1 Department of Infectious Diseases, University Medical Centre Ljubljana, 2 Institute for Biostatistics and Medical Informatics, Faculty of Medicine Ljubljana, 3 Institute of Microbiology and Immunology, Faculty of Medicine Ljubljana, Ljubljana, Slovenia

**Background** *Borrelia burgdorferi* sensu stricto, the causative agent of Lyme borreliosis (LB) possesses a functional 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGR), which is a rate limiting enzyme of the mevalonate pathway that contributes to cell wall synthesis. Statins are HMGR inhibitors and have been shown to reduce bacterial burden and alter the immune response to favour clearance of spirochetes in a mouse model of LB.

**Methods** The association between background statin use and clinical and microbiologic characteristics was investigated prospectively in 1220 adult patients with early LB manifesting as erythema migrans (EM) at a single-centre university hospital. Patients were assessed at enrolment and followed-up for 12 months.

**Results** Statin treatment was associated with age and prevalence of other comorbidities besides hyperlipidemia, but not with borrelial skin culture positivity rate, serological response to infection, or presence of LB-associated symptoms at enrolment. The proportion of patients taking statins was lower among patients with disseminated disease manifested as multiple EM than among those with solitary EM, but the difference was nonsignificant (10/195, 5.1% vs 84/1025, 8.2%; $P$=0.19). The time to resolution of EM after starting antibiotic treatment was comparable in patients on statins and in those without statins (median 7 days, IQR 4–14). At 12 months, 59/989 (6.0%) patients showed incomplete response. The odds for incomplete response decreased with time from enrolment (odds ratio (OR) 0.49, 0.50, and 0.48 for 2-month vs. 14-days, 6-month vs. 2-month, and 12-month vs. 6-month follow-up visits, respectively), were higher in patients who reported LB-associated constitutional symptoms at enrolment (OR 8.10, 95% CI 5.69–11.55; $P$<0.001), but were not affected by statin use (OR 1.09, 95% CI 0.61–1.98; $P$=0.76).

**Conclusions** In our study of European patients with EM, most of whom were infected with *B. afzelii*, statin use was not associated with selected clinical and microbiological parameters of infection.

A14 **Candidatus Neoehrlichia mikurensis in febrile patients and ticks in the Alsace region of France**

Pierre H. Boyer 1; Laurence Zilliox 2; Lisa Baldinger 2; Marine Engel 2; Xavier Wirth 1; Chasy Mewa Kamdem 1; Yves Hansmann 1, 3; Nathalie Boulanger 1, 2; Benoît Jaulhac 1, 2

1 EA 7290: Early Bacterial Virulence: Borrelia Group, CHRU Strasbourg, Fédération de Médecine Translationnelle de Strasbourg, F-67000 Strasbourg, France, 2 French National Reference Center for Borrelia, Hôpitaux Universitaires de Strasbourg, Strasbourg, France, 3 Department of Infectious and Tropical Diseases, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

**Background** *Candidatus* Neoehrlichia mikurensis (CNm) has recently been identified as a relevant tick-borne pathogen causing high fever as main symptom; thromboembolic events were also reported. Immunocompromised but also apparently immunocompetent patient have been described so far.

We investigated for its presence in patients presenting with fever after a suspicion of tick bite and also in *Ixodes ricinus* tick vector.

**Methods** DNA extracts from whole blood samples of 53 febrile patients sent to our laboratory between January 2018 and September 2018 were studied. All patients had developed a fever above 38 °C after a known or suspected tick bite in the Alsace region of France. In parallel, 512 *I. ricinus* questing nymphs collected by flagging from March to June 2017 in four separate locations in Alsace, an endemic area for Lyme borreliosis, were investigated.

Both patients and ticks were tested for CNm using an in-house Taqman® real-time PCR assay, targeting a conserved region of the *groEL* gene of CNm.

**Results** Among the 53 tested patients, two were found positive for CNm. Both were symptomatic and had high fever, chills, malaise and muscle pain. The first patient had a chronic lymphoid leukemia and was splenectomized.

Notably, the other patient was not immunocompromised (immune lab testing performed *a posteriori* did not reveal any abnormality).

On the 512 tested ticks, 11 (2.15%) were positive for CNm, which is in line with the prevalence of other tick-borne pathogens than *Borrelia* observed in the region (A*naplasma phagocytophilum* and *Borrelia miyamotoi*)

**Conclusion** We described for the first time two clinical cases of CNm infection in France. These two clinical cases show that CNm infection can occur in immunocompromised as well as in immunocompetent patients. This latter pathogen should be included in the differential diagnosis of post-tick bite febrile syndromes.

A15 **Immune-mediated versus infective polyarthritis in dogs – do tick-borne pathogens play a role?**

Katalina Staufer, Michael Reiter, Anna Schötta, Gerold Stanek, Georg Duscher and Michael Leschnik

Klinische Abteilung für Interne Medizin Kleintiere, Veterinärmedizinische Universität Wien / Zentrums für Pathophysiologie, Infektiologie und Immunologie, Institut für Hygiene und Angewandte Immunologie, Medizinische Universität Wien / Veterinärparasitologie, Veterinärmedizinische Universität Wien

Immunmediated polyarthritis (IPA) is a quite frequent disease in dogs which is characterized by non-infective inflammation of several joints caused by pathological immune response. Several factors like genetic predisposition, environmental factors, chronic disease and neoplasia are mentioned as probable triggers. Tick-borne pathogens (TBP) might be either directly causing disease by joint infection or act as a trigger for immune-mediated conditions, too.

We examined client-owned dogs with polyarthritis and tested blood samples for antibodies against *Borrelia burgdorferi* senu lato (Western Blot), *Babesia canis* (indirect immunofluorescent assay), *Rickettsia* spp. (*R. slovaca, R. raoultii, R. helevtica, R. monacensis, R. massiliae*, IFA), *Anaplasma* spp. and *Ehrlichia* spp. (Idexx 4DX®). PCR targeting *Rickettsia* spp., *Babesia* spp., and *Borrelia* spp. were also performed from blood and synovia samples. IPA classification was performed according to common criteria.

In 9 out of 13 dogs with suspected IPA type 1 (no evidence for underlying systemic or organic disease) antibodies against at least one tick-borne pathogen could be detected. All these dogs had a positive antibody titer against *R. helvetica*. Antibodies to *Anaplasma* spp. and *Borrelia burgdorferi* were additionally positive in two respectively one dog and another dog had antibodies against *R. slovaca*, *R. raoultii* and *R. monacensis*. PCR for *Rickettsia* spp., *Babesia* spp., and *Borrelia* spp. were all negative in 12 dogs and one dog tested positive for *R. raoultii* in the synovial sample.

As expected, our findings indicate that dogs with IPA have a high exposure risk to TBP, interestingly mainly to *Rickettsia* species which could act as additional trigger for immune-mediated disease. Comparing these results with healthy dogs will clarify whether contact to TBP increases the risk to develop IPA. To our knowledge this is the first time that *Rickettsia* DNA could be detected in a synovia sample in a dog with polyarthritis.

A16 **Informing or scaring: predictors of tick bite anxiety and avoidance of green spaces.**

Ormel FF 1; Tulen AD 1;, Kosterman K 1; Wong A 2; Timmermans DRM 1, 3; Visser LG 4; van den Wijngaard Cees C. 1; Timen A 1

1 National Institute of Public Health and the Environment (RIVM), Centre for Infectious Disease Control, Bilthoven, The Netherlands, 2 National Institute of Public Health and the Environment (RIVM), Centre for Nutrition, Prevention and Health Care, Bilthoven, 3 Department of Public and Occupational Health, EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, The Netherlands., 4 Department of Infectious Diseases, Leiden University Medical Center, the Netherlands.

Background: Anecdotal observations suggest a high level of anxiety for tick bites in the Dutch population, after years of intensified public education regarding Lyme disease prevention. The aim of this study was to investigate the level of anxiety for tick bites and Lyme disease in the Dutch population, and to explore its predictors.

Methods: At two time points, an online questionnaire on anxiety, perceptions, knowledge, tick exposure and practices regarding tick bites and Lyme disease was sent to members (≥18 years) of a representative online panel. The sample was randomly divided into an intervention group that watched educational animations on Lyme disease, and a control group. Prediction models were used to identify predictors for anxiety and avoidance behavior, such as age, sex, knowledge and perception of severity of tick bites and Lyme disease, and the intervention.

Results: 1,025 respondents were included (504 in the intervention and 521 in the control group). 89.3% (n = 465) of the controls experienced anxiety when imagining having a tick bite, of which 33.4% (n = 174) experienced a high level of anxiety. 9% (n = 92) of the total population reported avoidance behavior (avoidance of wooded areas or keeping a child away from school trips). Perception of a higher severity of a tick bite best predicted the level of anxiety, as well as avoidance behavior. A history of tick bites lowered the anxiety level. The educational intervention was neither a good predictor for the level of anxiety, nor for avoidance behavior.

Conclusions: One third of the Dutch population experiences a high level of anxiety when they imagine being bitten by a tick. Rather than the investigated educational intervention, people's perception of the severity of a tick bite is a good predictor for anxiety, as well as for the avoidance of green spaces.

A17 **Borrelia miyamotoi Meningitis in One Immunocompetent and One Immunocompromised Individual – Report of Two Cases Diagnosed in Sweden**

Anna J Henningsson (A, B, C), Hilmir Asgeirsson(D, E), Berit Hammas(F), Elias Karlsson(G), Åsa Parke (D), Dieuwertje Hoornstra(H), Peter Wilhelmsson (A, B), Joppe W Hovius(H)

A: Clinical Microbiology, Division of Laboratory Medicine, Region Jönköping County, Sweden, B: Division of Medical Microbiology, Department of Clinical and Experimental Medicine, Linköping University, Sweden, C: Clinical Microbiology, Region Östergötland, Linköping, Sweden, D: Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden, E: Division of Infectious Diseases, Department of Medicine, Huddinge, Karolinska Institute, Stockholm, Sweden, F: Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden G: Department of Infectious Diseases, Danderyd University Hospital, Stockholm, Sweden, H: Center for Experimental and Molecular Medicine, Academic Medical Center, University of Amsterdam, The Netherlands

*Borrelia miyamotoi* is a relapsing fever borrelia transmitted by hard ticks. Human *B. miyamotoi* disease has been described from Russia and the USA, as a systemic illness causing relapsing fever, headache, myalgia, elevated liver enzymes, neutropenia and thrombocytopenia. So far, three cases of meningoencephalitis have been reported worldwide, two of them from Europe, one from the USA, and all in highly immunocompromised patients. In August 2018, two cases of *B. miyamotoi*-associated meningitis were diagnosed in Sweden, one in an immunosuppressed and one in an apparently immunocompetent patient. We describe the clinical symptoms and signs in the two patients, including molecular detection, phylogenetic analyses and serological responses.

The immunosuppressed patient was a 66-year-old woman with rheumatoid arthritis treated with rituximab and methotrexate. She presented with six weeks of intermittent high-grade fever and nine months of various other symptoms. Her symptoms debuted in November 2017 with headache and increasing fatigue. She subsequently experienced progressing hearing loss, memory and concentration difficulties. A lumbar puncture performed in August 2018 revealed mononuclear pleocytosis and 16S rRNA sequencing of the CSF suggested *B. myiamotoi*.

The immunocompetent patient was a healthy 53-year-old woman. Her sole medication was oxycodone which she was taking due to a recent elbow fracture. She had noticed a tick bite six weeks earlier and presented with headache, neck stiffness and high-grade fever that had progressively worsened during the last week. CSF analysis showed mononuclear pleocytosis and 16S rRNA sequencing of the CSF suggested *B. myiamotoi*.

The presence of *B. miyamotoi* was confirmed both in CSF and in serum from both patients using qPCR targeting the flagellin gene. Sequencing of *glpQ, p66* and a fragment of the 16S-23S rRNA intergenic spacer from both CSF samples revealed sequences identical to other *B. miyamotoi*-sequences derived from Europe. *B. miyamotoi* infection was also confirmed serologically. Both patients recovered after doxycycline treatment.

A18 **Experimental infection with the Lyme disease pathogen in the rodent host dramatically changes the microbial community in blood-feeding ticks**

Phineas Hamilton 1, Anouk Sarr 2, Elodie Maluenda 2, Cindy Bregnard 2, Olivier Duron 3, Olivier Plantard 4, and Maarten J. Voordouw 1, 5

1 Deeley Research Centre, BC Cancer, Victoria BC, Canada, 2 Institute of Biology, University of Neuchatel, Neuchatel, Switzerland, 3 Laboratoire MIVEGEC, Centre National de la Recherche Scientifique (CNRS), Montpellier, France, 4 BIOEPAR, INRA, Oniris, 44307, Nantes, France, 5 Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada

Tick-borne diseases remain an important global public health problem. Interactions between tick-borne pathogens and the microbial community in the tick midgut can have important consequences for both partners. To date, few experimental studies have investigated the nature of the interactions between spirochete bacteria of the *Borrelia burgdorferi* sensu lato (sl) genospecies complex, which include the causative agents of Lyme disease, and the microbiome of their *Ixodes* tick vectors. We used a large-scale experiment to clarify the reciprocal interactions between *Borrelia afzelii* and the microbiome of *Ixodes ricinus*, its primary vector. Adult female *I. ricinus* ticks that had engorged on roe deer were allowed to lay eggs in the lab. Egg clutches were split in half and washed with water (control) or bleach (dysbiosis); the bleach treatment reduced the bacterial microbiome 30-fold in the hatched larvae. Control larvae and dysbiosed larvae were allowed to feed on uninfected control mice (n = 20) or mice experimentally infected with *B. afzelii* (n = 20). The blood-engorged larvae were allowed to molt into nymphs, of which 400 were tested for *B. afzelii* infection, microbial abundance, and the microbial community. The 30-fold reduction of the bacterial microbiome in the larval ticks had disappeared in the nymphs and there was no effect of the microbiome depletion (following bleach treatment of eggs) on the subsequent probability of acquisition of *B. afzelii* by ticks during blood feeding. Infection status of the mouse caused dramatic changes to the tick microbiome, decreasing bacterial abundance, shifting community composition, and increasing diversity. These strong effects suggest that tick blood meals obtained from vertebrate hosts infected with *B. burgdorferi* sl have dramatic consequences for other tick-associated microbes or pathogens.

A19 **The search for Borrelia and other tick-borne pathogens down under**

Charlotte Oskam, Siobhon Egan, Siew-May Loh, Alexander Gofton, Telleasha Greay, Una Ryan and Peter Irwin

Vector and Waterborne Pathogens Research Group, Murdoch University, Perth, Australia

Ticks (Acari: Ixodida) are blood sucking arthropods and transmit the greatest variety of pathogenic microorganisms of medical and veterinary importance. During the past decade, a third of world-wide pandemics have been associated with bacterial and protozoal emerging infectious diseases and over a quarter have been attributed to vector-borne diseases.

Australia is home to 68 native and five introduced tick species. While most have native wildlife hosts, only a few tick species are known to bite humans and to vector pathogens associated with human tick-borne diseases. However, the concern regarding the potential for zoonotic tick-borne illness in Australia has increased which lead to a recent Parliamentary enquiry to address public concern about ticks and tick-borne disease. Therefore, the question of a novel tick-borne zoonotic disease acquired from Australian ticks is of critical scientific and political importance, and it is a conundrum that requires urgent research to provide evidence-based scientific data.

This presentation will discuss the methodology used to characterise the bacterial communities found within Australian ticks and the search for novel candidate pathogens, as well as bacterial genera of known tick borne pathogens and endosymbionts. Bacterial endosymbionts are not known to be infectious to vertebrate hosts, however this principle has been challenged recently and the distinction between arthropod endosymbionts and vertebrate pathogens has become blurred. Our recent findings show that without the use of molecular advances, such as next-generation sequencing, many bacteria of potential medical and veterinary interest could go undetected.

A20 **Tick eggs as a source for the whole genome sequencing of transovarially transmitted Borrelia miyamotoi**

Markéta Nováková[1], Jan Janeček[1], Petra Pospíšilová[1], Li Dantong[2], Ana Silva[3], Anita Cunha[3], Alena Žákovská[4], David Šmajs[1*]

[1]Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic,
[2]Southern Medical University, Guangzhou, China,
[3]Faculty of Medicine, University of Porto, Porto, Portugal,
[4]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Tick-borne pathogens exploit several ways in order to persist in nature. Lyme disease borreliae are maintained in amplifier hosts (e.g., in rodents). The relapsing fever *Borrelia miyamotoi* is known to perpetuate in rodents and is also transmitted transovarially in *Ixodes* spp. ticks. The latter ability increases its epidemiological potential, since non-engorged larvae may be a source of infection for vertebrates. We hypothesized that freshly laid tick eggs may represent a suitable material for the whole genome sequencing of transovarially transmitted bacteria since they contain less bacterial species and less tick DNA than a tick specimen.

We collected 266 engorged females of *Ixodes ricinus* from dogs and cats and one deer, maintained them at 26 °C and 95% relative humidity, obtained egg clusters from 158 ticks and isolated DNA by using Qiagen Blood & Tissue kit. The presence of *B. miyamotoi* DNA was confirmed by detection of the *glpQ* gene in 3 out of 158 egg clusters. The ratio of borrelial to tick DNA was evaluated by nested PCR (*glpQ* for *B. miyamotoi*, *ITS2* for tick DNA) and two samples (F1 and F190) with high proportion of borrelial DNA were sequenced by Illumina platform. Samples contained 78% and 54.1% of *Ixodes ricinus* DNA, 19.5% and 31.4% of *Candidatus* Midichloria mitochondrii DNA and 2.5% and 14.5% of *Borrelia miyamotoi* DNA, respectively. An average depth coverage reached 647x and 3,216x for F1 and F190 samples, respectively.

Detailed analyses of the genome sequence of *B. miyamotoi* obtained from *I. ricinus* ticks will reveal how this strain differs from strains harboured by other ixodid species in geographically distant regions and will contribute to the description of its evolutionary history.

A21 **Molecular analysis of variability among Borrelia miyamotoi strains from the Czech Republic**

Jan Janeček[1], Petra Pospíšilová[1], Markéta Nováková[1], Li Dantong[2], Ana Silva[3], Anita Cunha[3], Alena Žákovská[4], David Šmajs[1*]

[1]Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic,
[2]Southern Medical University, Guangzhou, China,
[3]Faculty of Medicine, University of Porto, Porto, Portugal,
[4]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Despite the widespread holarctic distribution of *Borrelia miyamotoi*, existing studies suggest a low genetic variability among Asian and North American strains.

In order to obtain information about genetic variability among Czech isolates of *B. miyamotoi* and to compare them with non-European strains, we analyzed ticks collected by flagging in four different localities in Moravia region forming the eastern part of the Czech Republic.

Since the prevalence rate of *B. miyamotoi* in *Ixodes ricinus* ticks is considered to be relatively low with a mean around 2%, a preselection of collected specimens was made. Obtained ticks were shredded to pieces and observed using dark-field microscopy for the presence of spirochetes. Only the ticks containing a visible amount of spirochetes were further processed by using DNA extraction followed by nested PCR specifically targeting the *glpQ* gene of *B. miyamotoi*. Thirty out of 635 examined ticks were positive for the presence of *B. miyamotoi* DNA.

Since the sequencing of the *glpQ* region revealed no variability among all 30 samples, we decided to analyze additional regions of the borrelial genome and compare them with those from non-European strains. We amplified and obtained sequences of eight highly variable intergenic spacer regions (IGS). These results also showed no differences among 30 Czech isolates and thus support a highly homogeneous genetic profile of *B. miyamotoi*. The phylogenetic tree constructed using all eight concatenated IGS sequences using maximum likelihood method placed the Czech isolates out of the clusters of both Asian and North American strains suggesting that despite the homogeneous background of the bacterium, separate lineages based on geographic distribution exist.

Another analyses using multilocus sequence typing of several borrelial housekeeping genes are currently in process to better analyze the phylogeny of Czech *B. miyamotoi* isolates.

A22 **Spirochaetal bacteria isolated from mosquitoes constitute a novel genus Entomospira genus novum within the order Spirochaetales**

Lucia Grana1#, Silvie Sikutova2#, Volker Fingerle3, Santiago Castillo-Ramirez1, Gabriele Margos3, Ivo Rudolf2

1 Programa de Genómica Evolutiva, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Apartado Postal 565-A, CP 62210, Cuernavaca, Morelos, México 2 Institute of Vertebrate Biology, v.v.i., The Czech Academy of Sciences, Kvetna 8, CZ-60 365 Brno, Czech Republic, 3 National Reference Center for Borreliosis at the Bavarian Health and Food Safety Authority, Veterinärstr. 2, 85764 Oberschleissheim, Germany, # authors contributed equally to this work

Several spirochetal bacteria were successfully isolated from mosquitoes (*Culex pipiens*, *Aedes cinereus*) in the Czech Republic between 1999 and 2002. Partial 16S rRNA sequence analysis showed that these strains differed from other spirochaetal genera in the family *Spirochaetaceae* and indicated that they may constitute a novel bacterial genus within this family.

To obtain more comprehensive data regarding the taxonomic positioning of these isolates, we have applied Illumina MiSeq and Oxford Nanopore technologies to sequence four genomes of these spirochaetes (namely strains BR151, BR149, BR193 and BR208) previously provisionally named '*Candidatus* Spironema culicis'. SPAdes v. 3.9.1. was used to assemble draft genomes which were further compared using Mauve v. 2.3.1.. In addition, draft genomes were compared to 36 publicly available genomes encompassing eight genera from the order *Spirochaetales*. A phylogeny was generated from orthologous gene sequences across all relevant taxa. The percentage of conserved proteins (POCP) was used to determine the genus status of these novel spirochaetes.

The overall size of the genomes varied between 1.67 Mb to 1.78 Mb; the GC content ranged from 38.5 % to 45.76 %. In phylogenetic analysis, the four isolates formed a cluster separate from all other taxa in the family *Spirochaetacea*e. Strains BR151 and BR149 were closely related whilst the two other isolates appear to form different species. POCP analysis revealed that these novel spirochaetes species did not belong to any previously recognized genus and thus, the genus *Entomospira* gen. nov. was proposed. BR151 was selected as type species for the genus *Entomospira* gen. nov. For this isolate and the closest related isolate, BR149, we propose the name *Entomospira culicis* sp. nov.

A23 **Borrelia-induced inhibition of antigen presentation through RIPK2-mediated intracellular signalling impaired functional T cell responses to Candida albicans**

M.A.E. Brouwer, W. Jones-Warner, S. Rahman, M. Kerstholt, M. Oostinga, G.J. Hooiveld, M.G. Netea, L.A.B. Joosten

Department of Internal Medicine, Radboud Center for Infectious Diseases (RCI), Radboud Insitute of Molecular Life Sciences (RIMLS), Radboud University Medical Center, 6525 GA Nijmegen, The Netherlands, Nutrition, Metabolism, and Genomics group, Division of Human Nutrition, Wageningen University

Antigen presentation is a crucial mechanism present in innate immune cells that instructs adaptive immune cells. Loss of this pathway severely impairs the development of the adaptive immune response. To investigate how Borrelia spirochetes modulate the induction of an effective adaptive immune response, primary human PBMCs were isolated from healthy volunteers and stimulated with Borrelia burgdorferi for 24 hours. One of the major findings was the suppression of the antigen presentation machinery. Through cell entry and RIPK2 signalling cascades, Borrelia burgdorferi strongly downregulated genes and proteins involved in antigen presentation, specifically HLA-DM, MHC class II and its chaperone CD74. Antigen presentation proteins were distinctively inhibited in CD14+ monocyte subsets, monocyte-derived macrophages and monocyte-derived dendritic cells. When compared to a range of other microbial pathogens, Borrelia-induced suppression of antigen presentation was specific for the pathogen. Inhibition of antigen presentation interfered with T cell recognition of Borrelia, even following re-stimulation of PBMCs with the commensal microbe Candida albicans, resulting in reduced T cell function through decreased IFN-γ, IL-17 and IL-22 production. These findings may explain why patients with Lyme disease develop a belated adaptive immune response during Borrelia infection. By discovering the exact mechanism of Borrelia-induced inhibition of antigen presentation via cell entry and RIPK2 signalling cascades, the failure of instigating a robust adaptive immune response against Borrelia spirochetes in Lyme Disease may be explained. This knowledge may be implemented to develop improved diagnostic methods and target based treatment strategies for Lyme patients in the future.

A24 **Profiling the Early Interaction Between Human Dendritic Cells and the Causative Agent of Lyme Disease Borrelia burgdorferi**

Maria G. Gutierrez-Hoffmann, Eleni Tiniakou, Erika Darrah and Mark J. Soloski

Lyme Disease Research Center, Johns Hopkins University School of Medicine

Dendritic cells are the primary antigen presenting cells responsible for the initiation of T cell responses against invading pathogens. This process requires antigen capture, processing, and peptide presentation by dendritic cells to naïve T cells; engagement of co-stimulatory molecules on both cell types; and the expression of cytokines that polarize naïve T cells to a particular effector phenotype.

*Borrelia burgdorferi*, the causative agent of Lyme disease, likely encounters resident dendritic cells at sites of infection in the skin of human hosts. Yet, the initial interactions between *B. burgdorferi* and dendritic cells are poorly understood. To address this, we cultured live *B. burgdorferi* with human dendritic cells from healthy donors, profiled *Borrelia*-derived peptides presented using LC/MS/MS and identified novel as well as previously known peptides that are naturally presented by human dendritic cells. We also examined the expression of key co-stimulatory molecules in dendritic cells required for T cell activation and identified PD-L1 as an inhibitory signal that may hamper effective T cell activation by mature dendritic cells. Lastly, we profiled the cytokine milieu secreted by dendritic cells when exposed to live spirochetes and found robust secretion of the pro-inflammatory cytokines TNF-a and IL23p40, and the anti-inflammatory cytokine IL-10. Future experiments examining the functional capabilities of *B. burgdorferi* exposed dendritic cells will further our understanding of the adaptive immune response modulated by dendritic cells, and how this response may contribute to the varied clinical outcomes of patients with acute Lyme borreliosis.

A25 **Borrelia mayonii resists complement-mediated killing by inhibiting activation of the alternative and terminal pathway**

Lea Walter, Valerie Sürth, Florian Röttgerding, Peter F. Zipfel, and Peter Kraiczy

Institute of Medical Microbiology and Infection Control, University Hospital of Frankfurt, Paul-Ehrlich-Str. 40, D-60596 Frankfurt, Germany Department of Infection Biology, Leibniz-Institute for Natural Products Research and Infection Biology, Beutenbergstr. 11a, D-07745 Jena, Germany

*Borrelia (B.) mayonii* has recently been identified as a novel human pathogenic genospecies causing Lyme disease in North America. Current data reveals a higher spirochaetemia in the blood of patients compared to individuals infected by *B. burgdorferi,* suggesting that this novel genospecies exploit strategies to overcome innate immunity, in particular complement. In order to elucidate the molecular mechanisms, we sought to identify the key determinant(s) involved in the interaction with human complement. When incubating *B. mayonii* with 50% normal human serum, nearby all spirochetes were highly motile indicating that cells overcome complement-mediated killing. Furthermore, only a few spirochetes stained positive for activated complement component C3 and the terminal complement C5b-9 complex. Next, we sought to elucidate the role of the key alternative pathway regulators factor H (FH) and FHL-1 in immune evasion of *B. mayonii*. Serum adsorption experiments revealed that spirochetes acquire both complement regulators. Moreover, FH retained its factor I-mediated C3b-inactivating activity when bound to spirochetes. Using bioinformatics, we identified a gene exhibiting 60% identity to the *cspA* encoding gene of *B. burgdorferi*. The CspA orthologous protein CspA_Bmayo was analyzed regarding its complement binding properties as well as its inactivating capacity on the alternative, classical, and Lectin pathway. These functional analyses revealed that the CspA_Bmayo interacts with FH and FHL-1 and thereby blocks activation of the alternative pathway. In addition, CspA_Bmayo terminates assembly of the terminal complement complex C5b-9 by preventing C9 polymerization. To further assess the role of CspA_Bmayo in facilitating serum resistance, serum-sensitive spirochetes were transformed with a shuttle vector allowing expression of the CspA_Bmayo encoding gene under its native promotor. Spirochetes producing CspA_Bmayo on the cell surface overcome complement-mediated killing indicating that this molecule facilitates serum resistance of *B. mayonii*. In summary, this is the first report describing an immune evasion mechanism utilized by *B. mayonii.*

A26 **An overview of multiple studies on the emerging tick-borne pathogen Borrelia miyamotoi in Russia: 2009-2019**

Alexander Platonov, Olga Stukolova, Konstantin Mironov, Nadezhda Kolyasnikova, Konstatin Kuleshov, Valery Yakimenko, Alexandra Filkova, Denis Sarksyan, Olga Platonova, Joris Koetsveld, Joppe Hovius

Central Research Institute of Epidemiology, Moscow, Russia; Amsterdam University Medical Centre, the Netherlands

Human cases of *Borrelia miyamotoi* disease (BMD) were first described in Russia (Platonov et al, 2011). Since then clinical BMD cases were found in many regions of Eurasia and North America. In this presentation we will provide a comprehensive overview of our studies on *B. miyamotoi*.

We performed clinical and epidemiological studies with long-term follow-up. Laboratory diagnostics of BMD by PCR and serological methods were specially developed for this study. We also isolated clinical strains by culture and performed whole genome sequencing. This allowed us to develop Multilocus sequence typing (MLST) and antigen typing schemes of isolates from humans and ticks. We performed *in vitro* studies on bactericidal activity of human serum and/or neutrophils against *B. miyamotoi* and *B. miyamotoi* interaction with the blood coagulation system. Finally, we experimentally infected voles and determined spirochetemia and antibody responses.

We identified more than 430 BMD cases in 13 Russian regions. Typical presentation of BMD consisted of a flu-like illness with high fever, fatigue, mild thrombocytopenia and lymphopenia, increased blood ALT/AST levels. Recurrent BMD episodes were observed if antibiotic treatment was delayed. All twelve clinical *B. miyamotoi* strains had an “Asian" genotype and carried approx. 15 linear and circular plasmids. The more than 100 Russian *B. miyamotoi* DNA isolates belonged to 8 different MLST sequence-types, and expressed different Variable major proteins, most frequently Vlp-Delta. Patients developed robust IgM and IgG antibody response to these antigens and GlpQ. *B. miyamotoi* was sensitive to antibody-mediated complement-dependent lysis and could be phagocytized by neutrophils. *B. miyamotoi* enhanced clot formation via activation of the intrinsic coagulation pathway, but did not affect platelets activation.

BMD is widespread in Russia and poses a threat to the population in endemic regions. These studies were supported by the Russian Science Foundation (project 15-15-00072-П) and partly by Netherlands Organisation for Health Research and Development (project 522003007).

A27 **Medical assessment and follow-up of patients with chronic complaints and suspected tick-borne disases**

Randi Eikeland, on behalf of the Board/ editor group of the Nordic consensus working Group on Medical assessment and follow-up of patients with chronic complaints and suspected tick-borne diseases: Ingeborg Aaberge, Anna J Henningsson, Tone Synnestvedt, Svein H. Henrichsen, Knut Erik Eliassen, Marika Nordberg, Sigurdur Skarphedinsson, Kjerst Wedding

Norwegian National Advisory unit on Tick-borne Diseases, Sørlandet sykehus Norway

Background: Tick-borne diseases (TBD) are common in Europe. During the last three years, an expert group who has compared recommendations and clinical practices of assessment of borreliosis and tick-borne encephalitis (TBE) in the Nordic countries, has found them neglectedly different, and in agreement with updated European guidelines. The consensus group agreed that there is no need for new Nordic guidelines on the treatment of acute borreliosis and TBE. We also found that knowledge and diagnostic of new emerging TBD as such as anaplasmosis, rickettsiosis, bartonellosis, babesiosis, neoehrlichiosis is lacking, as are recommendations of assessment and follow-up on patients with chronic complaints after TBD, or suspected TBD -where no confirmed tick-borne infections are found.

Method: The Norwegian Directorate of Health formed a mandate to make a Nordic consensus for medical assessment and follow-up of patients with suspected TBD: Clarification and recommendations for care of persons where TBD are suspected, but where no active TBD are found. The patient's organizations and Norwegian Institute of Public Health must be included in the Nordic consensus working group. More than 40 experts from our four Nordic countries have worked on this since 2017. The work was divided in four work packages (WG):

WG 1: Literature search

WG 2: Development of an algorithm for assessment and follow-up

WG 3: Development of an algorithm for laboratory diagnostics

WG 4: Development of rehabilitation recommendations

The results were presented and discussed on an open consensus meeting in Oslo in October 2018.

Results: The process and the algorithms are currently under review and will be presented at the International Symposium on Tick-Borne Pathogens and Disease in Vienna.

A28 **Development of proteomics for the direct diagnosis of Lyme borreliosis**

P. Cantero, L. Ehret-Sabatier, B. Jaulhac, Y. Hansmann, C. Lenormand, P. Kieffer, L. Zilliox, N. Boulanger

1 Laboratoire de Spectrométrie de Masse BioOrganique, Institut Pluridisciplinaire Hubert Curien (UMR 7178), CNRS-Université de Strasbourg, France 2 EA7290 : Virulence bactérienne précoce : groupe Borrelia, Université de Strasbourg, France, 3 Centre National de Référence Borrelia, Centre Hospitalier Régional Universitaire, Strasbourg, France 4 Service d'Infectiologie, CHRU Strasbourg, France, 5 Clinique Dermatologique, CHRU Strasbourg, France, 6 Service de Médicine Interne, Centre Hospitalier de Mulhouse, France

The diagnosis of Lyme borreliosis currently relies mainly on indirect detection of *Borrelia burgdorferi* sensu lato (sl) antibodies by ELISA ± western-blot. However, this diagnostic technique is not fully satisfactory for patients since it does not prove an active infection, especially in disseminated infection with multiple and diverse clinical manifestations.

To improve the direct diagnosis of Lyme borreliosis, a targeted proteomic approach using Mass Spectrometry (SRM-MS) was developed. Since *Borreliae* are inoculated in the skin, multiply locally and then can spread to different organs and since some persist in the skin for months, a SRM-MS method was first developed to identify *Borrelia* proteins in the skin of *Borrelia*-infected mouse. Then, the technique was tested in patients (erythema migrans biopsies) as a proof of concept with identification of two *Borrelia* proteins in the human skin, OspC and flagellin1, as relevant targets. We are currently in the process of validating this mass spectrometry-based diagnosis tool on a cohort of 70 patients by comparing to other direct diagnosis techniques, *Borrelia* culture and PCR (*fla* gene).

In this context, targeted proteomics enables the direct detection of up to four *Borrelia* proteins in patients, as well typing of *Borrelia* species thanks to species-specific peptides. The technique appears to be more sensitive for 30% of biopsies analyzed up to now: it can detect more pathogen markers (proteins) in infected tissues than PCR which usually targets only one (flagellin gene).

In conclusion, the detection of bacterial proteins using mass spectrometry has the advantage of a better sensitivity and a multiplexing capacity. These results are promising for the improvement of the direct diagnosis of disseminated Lyme borreliosis2 and it might be extended to other tick-borne diseases.

1Schnell et *al*., Mol. Cell. Proteomics 2015, 14: 1254-1264
2 Grillon et al., Scientific reports 2017, 7:16719

A29 **Standardization and quality control of Antibody detection for Lyme Borreliosis. One assay for all - one ring to rule them all?**

Ram B. Dessau

Department of clinical microbiology, Slagelse Hospital, Region Zealand, Denmark

“Lack of standardization" for Borrelia serology is often stated in both scientific presentations and publications, with the implicit understanding, that this should be of course be done. However, the presenters rarely follow up on the issue, proposing what should be standardized and which methods to use. There is also lack of a critical discussion, if this would at all be possible? There are a lot of different commercial products on the market using different proprietary antigens and new assays are turning up frequently. Evaluation is often scarce and restricted to a laboratory comparison with another assay, without the clinical aspect. Biological variation is the primary challenge to standardization of complex biological molecules and their interaction. In principle antibody development to different antigens in different people exposed to different strains of *Borrelia sp.* is a dynamical and random process concerning both detectable levels of reactivity and the temporal development to borrelial antigens. Large heterogeneity among studies of diagnostic accuracy has been documented (Leeflang et al. 2016).

There are two areas to consider. How to conduct external quality control and the evaluation diagnostic accuracy.

Concerning external quality control it would be important to separate the analytical within assay performance, from the between assay performance. Present day EQC (“round robin") mix up the analytical aspect with the clinical interpretation. The challenge here is to set the criteria for successful outcome of the clinical interpretation and the rare occurrence of borrelia-manifestations.

Concerning validation and evaluation of diagnostic accuracy the choice of patient and control groups is very essential – and is a challenge due to several issues such as sample collection. The reference standard is clinical and not independent of the serology to be evaluated.

These issues will be presented and possible compromise solutions will be discussed.

A30 **Serologic risk-stratification by a novel C6-peptide IgG1subclass antibody test**

Dag Nyman, Marika Nordberg, Susanne Olausson, Sten-Anders Carlsson

Åland Group for Borreliosis Research

The diagnosis of borreliosis relies still on demonstration of positive IgG-antibodies specific antibodies in serum. The diagnostic performance of existing antibody assays is questionable, mainly due to a high background seropositivity. Therefore, the main application of antibody analysis is risk stratification. An efficient risk stratification requires approximately 100 % sensitivity with preserved maximal specificity set by the background level of seropositivity. There is still a need for an optimal assay. The IgG-immunoresponse is spread over 4 subclasses with different kinetic and dynamic properties.

We have explored the use of the IgG1-subclass antibodies to the borrelia C6-peptide as a diagnostic test. An ELISA for detection of mentioned antibodies was set up. In 473 consecutive patients from the diagnostic routine IgG1 *Borrelia* antibodies were determined in parallel with a combinate diagnostic algorithm using C6-peptide total antibodies IgM/IgG (Immunetics) and IgG ELISA (recomWell Mikrogen) verified by bead-based IgG antibody assay (recomBead on Luminex, Mikrogen).

The correlation coefficient between C6-IgG1Ab and C6IgG/M totalAb was 0.9486, $p<0.0001$. In 310 healthy blood donors the correlation coefficient was 0,9185, $p< 0.0001$. The difference between the correlation coefficients of patients and blood donors was significant, $p<0.0012$. The fraction of seropositivity was for C6-totalAb 53.3 % and for C6IgG1Ab 59.8 %, p for difference = 0.044, in the patient cohort and in the blood donors C6-totalAb 27.7 % and 36.1 % for IgG1AB in blood donors, p = 0.025.

The bench performance for the C6-IgG1 assay is in favor of increased sensitivity as required for a risk-stratification test.

Funded by grants from the foundation of medical research of the Åland Cultural Foundation and from the Wilhelm and Else Stockmann Foundation.

A31 **Validity of the LTT as diagnostic and follow-up marker for Lyme neuroborreliosis**

Gert Summer1, Alexander Spoerl2, Volker Fingerle3, Christian Lechner1,2, Tobias Armin Rupprecht1

1Department of Neurology, Helios clinic Munich-West, 2Department of Neurology, Helios clinic Dachau, 3National reference Center for Borrelia, Oberschleissheim, Germany

A key issue of our study was assessing the validity of a commercially offered Lymphocyte-Transformation-Test (LTT), regarding diagnosis of Lyme-Neuroborreliosis (LNB).

In summary, LTT is based on measuring the proliferative rate of peripheral blood T-cells, which are incubated with different lysate *Borrelia* antigens compared to stimulation with control substances. The development of LTT relies on some methodically questionable studies, suggesting that borrelia might sometimes escape from sufficient activation of the B-cell-system. Due to these studies, LTT could be capable of detecting antibody negative Borreliosis. Though LTT is already frequently used as a diagnostical test in the clinical routine and as a tool for therapeutical decision-making, there is no convincing scientific evidence for using LTT so far, due to a lack of well designed and reliable prospective clinical studies.

Therefore we conducted a prospective dual centre study from 05/14-11/17. We compared patients suffering from confirmed LNB (according to the EFNS-guidelines) with other inflammatory CNS-diseases: Bell's palsy, viral meningitis, herpes zoster, guillan-barre-syndrome, and multiple sclerosis. We enrolled a total of 110 study participants and LTT was evaluable in 70,9% (15 persons in the LNB group and 63 persons in the control group). After all, we found only a poor sensitivity of 53,3% and low-grade specificity of 84,1% for LTT, referring to the detection of LNB. Furthermore, the LTT-levels 3 months after a sufficient antibiotic therapy did not correlate with the therapeutic response of LNB patients. This indicates that LTT cannot serve as a follow-up marker.

Thus we conclude, that LTT is neither appropriate for detecting LNB nor should it be used to estimate sufficiency of antibiotic therapy.

A32 **Tick global, act local. Emergence and elimination of a Rickettsia massiliae-infected Rhipicephalus sanguineus population in Central-Europe**

Gábor Földvári1, 2, Sándor Szekeres2, Gábor Majoros2, Alexandra Juhász2, Míra Mándoki3, Sándor Hornok2, Éva Fok2, Hein Sprong4

1Evolutionary Systems Research Group, Centre for Ecological Research, Hungarian Academy of Sciences Tihany, Hungary +3670 379 7192 FoldvariGabor@gmx.de, 2Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary 3Department of Pathology, University of Veterinary Medicine, Budapest, Hungary, 4Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and Environment, Bilthoven, the Netherlands

In July 2015 we received a large number of equally-fed nymphal ticks from a mixed-breed dog. In August, many equally-fed nymphs were removed from a beagle. Both dogs originated from the same kennel of a dog shelter near Budapest. They moulted to adults in the laboratory and were identified as *R. sanguineus* both with morphological and molecular tools. During the site visit large number of larvae, nymphs and adults were observed alive in and around the affected kennel. Although the kennel was treated with bleach and pesticide, ticks were all over the walls, floor, fence and cracks. The three puppies placed in the kennel were also infested. Over one thousand ticks were collected from all stages (mainly adults), but we estimated the tick population size of several ten thousands. Flagging around the shelter and at the site of capture of the first infested dog did not detect *R. sanguineus*. We could not identify the exact origin of the imported population but based on the molecular results, introduction from Italy is possible. *Rickettsia*-specific qPCR performed on blood samples of the affected asymptomatic dogs was negative but yielded positive in 68% of the 184 ticks examined. Sequencing identified the human pathogenic *Rickettsia massiliae*. As previous pesticide treatments failed and the dog shelter's budget was limited, we advised controlled burning by flamethrower (gas torch). Living ticks could be observed still after the first treatment with flame. The second flame treatment finally resulted in elimination of the *R. sanguineus* population in September. Here we attempted to apply the general protocol, DAMA (Document–Assess–Monitor–Act), which is an integrated proposal to build a proactive capacity to understand, anticipate, and respond to the outcomes of accelerating environmental change.

A33 **A phase 1 study assessing the safety, immunogenicity and dose-response of VLA15, a multivalent recombinant OspA based vaccine candidate against Lyme borreliosis, in healthy adults aged below 40 years**

Nicole Bézay1, Romana Hochreiter1, Vera Kadlecek1, Andreas Pilz1, Isabel Leroux-Roels2, Geert Leroux-Roels2, and Katrin Dubischar1

1 Valneva Austria GmbH, Campus Vienna Biocenter 3, 1030 Vienna, Austria, 2 Center for Vaccinology (CEVAC), Ghent University Hospital, 9000 Ghent, Belgium

**Background** Lyme borreliosis (LB) is the most common tick-borne disease caused by several genospecies of the spirochete *Borrelia burgdorferi sensu lato*. Increasing case numbers and lack of effective preventive measures emphasize the need for a vaccine. OspA is a dominant surface protein expressed by the spirochetes while in the tick midgut and a proven target for a LB vaccine. VLA15 is a subunit vaccine targeting the six most prevalent OspA serotypes that cause LB in Europe (OspA ST1-6) and the US (OspA ST1).

**Methods** We conducted a partially randomized, observer-blind, multi-centre Phase 1 study. A total of 179 healthy volunteers, age range 18-39 years, received three intramuscular immunizations, one month apart, of 12µg, 48µg or 90µg VLA15 formulated with alum or non-adjuvanted (six treatment groups). The study assessed the safety, immunogenicity (OspA IgG ELISA) and dose response of VLA15. Safety and immunogenicity of a booster dose applied 12-15 months after the first immunization were assessed in a subset of subjects.

**Results** VLA15 was safe and well tolerated in all dose groups and formulations, with very few severe related AEs. The majority of AEs were mild or moderate. Most common solicited local reactions were pain and tenderness, most common solicited systemic reactions were headache, fatigue and myalgia. No cases of arthritis or rheumatoid arthritis, AEs potentially associated with LB, were observed. VLA15 was immunogenic for all OspA serotypes in all doses and formulations. Immune responses were higher in the adjuvanted formulations compared to the non-adjuvanted formulations, and in the higher dose groups. A booster dose applied approximately one year after the first immunization elicited a significant increase in antibody titers compared to peak levels after primary immunization. Dose increase and an alternative schedule are investigated in an ongoing Phase 2 program in order to further optimize immune response.

A34 **Feeding-induced salivary gland genes of Ixodes ricinus ticks as targets for anti-tick vaccines**

Nayak A.1, Trentelman J.1, Hajdusek O.2, Klempa B.3, Krezdorn N.4, Anguita J.5, and J.W. Hovius1.

1Center for Experimental and Molecular Medicine, Academic Medical Center, Amsterdam, the Netherlands; 2Biology Center, Institute of Parasitology, Czech Academy of Sciences, Ceske Budejovice, Czechia; 3Biomedical Research Center, Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia; 4GenXPro GmbH, Frankfurt Main, Germany; 5Center for Cooperative Research in Biosciences (CIC bioGUNE), Derio, Spain.

In the ANTIDoTE project, a collaborative project on anti-tick vaccines funded by the European commission, we identified several vaccine candidates in salivary glands of independent *Ixodes ricinus* populations by implementing a transcriptomic approach that combines RNA sequencing and **m**assive **a**nalysis of **c**DNA **e**nds (MACE). Using DeSeq2 statistical analysis, we identified 3766 up-regulated and 3574 down-regulated genes, respectively. The top 25 genes that were significantly upregulated and highly abundant at 24 hours vs 0 hour post-feeding, were selected for further analysis and validation. Twenty genes were biologically validated since they demonstrated a significantly higher up-regulation at 24 hours compared to 0 hour as tested by quantitative real time PCR in another five independent cDNA pools derived from salivary glands of uninfected *I. ricinus* nymphs. Gene silencing (knockdown) of one of the top-20 candidates, GXP_Contig_8327 (a putative metalloprotease), in *Ixodes ricinus* nymphs demonstrated significant reduction in engorgement weights as compared to the control nymphs, post-feeding on mice. Additionally, in a lethal mouse model of tick-borne encephalitis a significant increase in survival rate of mice that were infected with nymphs (when GXP_Contig_8327 was silenced) harbouring tick-borne encephalitis virus, was observed. Interestingly, mice vaccinated with recombinant *E. coli*-expressed GXP_Contig_8327 and challenged with TBEV-infected nymphal ticks showed significantly more and longer survival, compared to control mice. Currently, other vaccine candidates in the list are produced as recombinant proteins and are planned to be tested in vaccination and challenge experiments in rabbit or guinea pig and mouse model to identify their potential role in tick immunity and pathogen transmission, respectively.

A35 **Identification of novel Ixodes vaccine candidates using Yeast Surface Display technology, going down the rabbit hole?**

Jos J.A. Trentelman1, Fons A. de Vogel1, Sukanya Narasimhan2, Erol Fikrig2, Jeroen Coumou1, Joris Koetsveld1, Jasmin I. Ersoz1, Ondřej Hajdušek3, Radek Šíma3, Juan Anguita4, Joppe W. Hovius1

Amsterdam UMC, University of Amsterdam, Center for Experimental and Molecular Medicine, Amsterdam, The Netherlands1, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, United States of America2; Biology Centre of the Academy of Sciences of the Czech Republic (BC ASCR), České Budějovice, Czech Republic3; Center for Cooperative Research in Biosciences (CIC bioGUNE), Derio, Spain4

*Ixodes* ticks transmit bacterial, protozoal and viral pathogens, causing disease and are 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 from 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. 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 FACS sorting using tick immune *I. scapularis* rabbit IgG. 2) MACS enrichment with IgG raised against 24h feeding *I. ricinus* nymphs followed by FACS sorting using tick immune *I. scapularis* rabbit IgG. Plasmids of isolated single yeast cells were sequenced to obtain the expressed protein sequences, resulting in the identification of 13 proteins that have highly conserved *Ixodes* epitopes and are likely to be involved in 'tick immunity'. These proteins could be excellent vaccination candidates targeting both *Ixodes ricinus* and *Ixodes scapularis* and their associated diseases.

Six vaccine candidates have been tested in rabbit vaccination studies, but did not show a significant effect on tick feeding parameters of nymphal and adult *I. ricinus*. However, preliminary data shows that one of these proteins greatly reduces transmission of *B. afzelii* in mice, without affecting tick feeding parameters, studies into the mechanism are currently being performed.

# Abstracts
# of presentations
# during the poster sessions

P1 **Clinical manifestations of early European Lyme neuroborreliosis**

Katarina Ogrinc (A), Stanka Lotrič Furlan (A), Petra Bogovič (A), Vera Maraspin (A), Tereza Rojko (A), Daša Stupica (A), Tjaša Cerar Kišek (B), Eva Ružić-Sabljić (B), Franc Strle (A)

A Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia, B Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Slovenia

**Background** Information on the frequency of different clinical manifestations of early European Lyme neuroborreliosis (LNB) is limited.

**Methods** This prospective study comprised 250 patients aged ≥ 15 years, diagnosed with early LNB between October 2005 and December 2018 at a single center. In all these patients LNB was attested with: i) CSF pleocytosis and ii) intrathecal synthesis of borrelial antibodies and/or the presence or reliable history of recent erythema migrans (EM). Borrelial infection was searched for in patients with no obvious other reason for their neurological signs/symptoms and who were characterized clinically with: radicular pain of recent onset (suspected Bannwarth's syndrome), EM associated with symptoms suggesting central nervous system involvement, or peripheral facial palsy (PFP) or other cranial nerve involvement.

**Results** There were 144 males and 106 females with median age 56 years. Most frequent clinical manifestation was meningoradiculitis (Bannwarth's syndrome) (149 patients, 59.6%), followed by peripheral facial palsy (PFP) or other cranial nerve involvement (62 patients, 24.8%) and borrelial meningitis associated with EM (39 patients, 15.6%). A reliable early LNB was established in 65.3% of patients with clinically suspected Bannwarth's syndrome, in 19.2% of patients with EM associated with constitutional symptoms suggesting CNS involvement, and in 7.9% of patients who presented with PFP. Median duration of neurological symptoms before diagnosis was 20 days. Objective signs found at presentation were PFP (44%), EM (35.2%), meningeal signs (16.8%), pareses (7.2%), tremor (2.4%), other cranial nerve involvement (2%), and borrelial lymphocytoma (0.8%). Intrathecal synthesis of borrelial IgM and IgG antibodies was present in 50.4% and 72.4% of patients, while borreliae (predominantly *B. garinii* from cerebrospinal fluid and skin, *B. afzelii* from blood) were isolated from cerebrospinal fluid, skin and blood in 8.8%, 32.6% and 1.3% of patients, respectively.

**Conclusions** Bannwarth's syndrome is the most frequent manifestation of early European LNB.

P2 **Lyme neuroborreliosis in children**

Mojca Rožič 1, Liza Lah 1, Eva Ružić-Sabljić 2, Andrej Kastrin 3, Maja Arnež 1

1 Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva ulica 2, 1000 Ljubljana, Slovenia, 2 Institute of Microbiology and Immunology Faculty of Medicine, University of Ljubljana, Zaloška cesta 4, 1000 Ljubljana, Slovenia, 3 Institute for Biostatistics and Medical Informatics, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia

**Background** Information on the etiology of Lyme neuroborreliosis (LNB) in children in Europe or North America is limited. There is no published study about the influence of *B. burgdorferi* sensu lato species isolated from cerebrospinal fluid (CSF) on clinical presentation of LNB in children.

**Methods** The study was monocentric during 17-year period. Children younger than 15 years with presentation suggestive of or confirmed Lyme borreliosis that had *B. burgdorferi* sensu lato isolated from CSF and had species of *B. burgdorferi* sensu lato identified by pulsed-field gel electrophoresis were included. Demographic and medical history data, data on clinical examination, neurologic examination, and laboratory results were gathered. This data was compared for children infected with *B. garinii* to those infected with *B. afzelii*.

**Results** 153 children had *Borrelia burgdorferi* sensu lato isolated from CSF. In 71/113 (62.8%) *B. garinii* and in 42/113 (37.2%) *B. afzelii* were identified. Altogether, 76.9% children had systemic symptoms and only 57,5% had positive meningeal signs. Compared to children infected with *B. afzelii*, children infected with *B. garinii* had erythema migrans less often (18.3% vs. 45.2%), but had positive meningeal signs (69.0% vs. 38.1%), lymphocytic predominance (97.1% vs. 75.0%), and elevated albumin CSF/serum quotient (80.6% vs. 50.0%) more often.

**Conclusions** In Slovenia, LNB in children is more often caused by *B. garinii*, followed by *B. afzelii*. When solely looking at the clinical picture and laboratory results, one cannot differentiate cases of LNB in children caused by *B. garinii* from those caused by *B. afzelii*.

P3 **Acute facial nerve palsy in children in a Lyme borreliosis-endemic area in the Netherlands**

Raitze A. Bruinsma 1, 2, C.A. Smulders 3, Y. Vermeeren 1, 4, B. van Kooten 1, 5, B. van Hees 1, 6, M. Boele van Hensbroek 7, J.W.R. Hovius 8, T.P. Zomer 1

1 Lyme Center Apeldoorn, Gelre Hospital, Apeldoorn, The Netherlands, 2 Department of Pediatrics, Gelre Hospital, Apeldoorn, The Netherlands, 3 Department of Pediatrics, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands, 4 Department of Internal Medicine, Gelre Hospital, Apeldoorn, The Netherlands, 5 Department of Neurology, Gelre Hospital, Apeldoorn, The Netherlands, 6 Department of Medical Microbiology and Infection Prevention, Gelre Hospital, Apeldoorn, The Netherlands 7 Amsterdam Medical Center, University of Amsterdam, Department of Pediatrics, Amsterdam, The Netherlands, 8 Amsterdam Medical Center, University of Amsterdam, Amsterdam Multidisciplinary Lyme borreliosis Center, Amsterdam, The Netherlands

Introduction: While facial palsy in children may be caused by Lyme neuroborreliosis (LNB), little is known about the prevalence of LNB in children with acute facial palsy in the Netherlands. This study aimed to determine the prevalence of LNB in children with facial palsy in a Lyme-endemic area of the Netherlands and to identify patient characteristics of children with facial palsy due to LNB.

Methods: We retrospectively reviewed patient records of all children ≤18 years who presented with acute peripheral facial nerve palsy (FNP) at three Dutch hospitals between January 2010 and December 2016, except for children with congenital facial palsy or recent surgery in the area of the facial nerve. LNB was defined as 'definite' if pleocytosis and intrathecal antibody production was present, 'probable' in case of pleocytosis with positive IgM serology or seroconversion, and 'possible' in case of only a positive IgM serology result (confirmed via immunoblot). Serologic testing was performed using an IgM and IgG ELISA followed by immunoblot. Pleocytosis was defined as leucocyte cell count in CSF >5 per µl. We calculated the overall prevalence of LNB and compared characteristics of children with facial palsy due to LNB (definite, probable, possible together) and idiopathic facial palsy (IFP).

Results: Of 104 included children (median age 7 years, 60% male), 41% (43) had LNB and 59% (61) IFP. LNB was diagnosed as definite in 22% (23), probable in 16% (17) and possible in 3% (3). Headache occurred in 55% (23/42) of LNB patients versus 19% (11/59) of IFP patients ($p<0.001$). Meningeal irritation occurred in 21% (9/42) of LNB patients versus 5% (3/59) of IFP patients ($p<0.05$).

Conclusion: Acute facial palsy in children is frequently caused by lyme neuroborreliosis. CSF should be examined in all children with facial palsy, especially in Lyme-endemic regions and particularly in case of headache.

P4 **A case of cervical myelitis as uncommon manifestation of neuroborreliosis in half-year monitoring**

Barbara Oczko-Grzesik, Witold Opiełka*, Krzysztof Tempka*, Barbara Sobala-Szczygieł, Damian Piotrowski, Lucjan Kępa, Wojciech Stolarz, Maciej Piasecki, Jerzy Jaroszewicz

Clinical Department of Infectious Diseases and Hepatology - Bytom, Medical University of Silesia, Katowice, Poland
*Department of Neurology, Specialist Hospital No. 1, Bytom, Poland

**Introduction:** Some atypical clinical manifestations of neuroborreliosis may cause the apropriate diagnosis and therapy delay.

**Aim:** The presentation of the course of cervical myelitis as unusual form of neuroborreliosis in half-year monitoring.

**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 diseaseand suspicion of chemical intoxication. MRI (outpatient-14.09.18): in the cervical section from C1/C2 to C5/C6 an abnormal region of elevated signal in T2-weighted 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:I gM–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 evident improvement of the patient's clinical state (with residue of periodic tremors of hands) and significant regression of CSF inflammatory parametrs. After half-year monitoring complete withdrawal of clinical symptoms and MRI as well as CSF parametrs abnomalities was found.

**Conclusion:** Lyme borreliosis should be considered in differential diagnostics of various neurological disorders.

P5 **Neuroborreliosis: retrospective analysis of hospitalised patients**

Alena Koščálová, Mária Sarvašová, Marek Kozák, Katarína Holečková

Departement of Infectology and Geographical medicine, Bratislava, Slovakia, Faculty of medicine of Slovak medical university, Bratislava, Slovakia, Faculty of medicine of Comenius university, Bratislava, Slovakia

**Background** The Lyme neuroborreliosis (LNB) is the infection of the nervous system by the spirochete *Borrelia burgdorferi* sensu lato. In adults, LNB mainly presents as lymphocytic meningoradiculitis (Bannwarth syndrome), in children as isolated facial nerve palsy or lymphocytic meningitis. Confirmation of production of anti-Borrelia antibodies in cerebrospinal fluid (CSF) is required for definite diagnosis of LNB.

The aim of this study was to evaluate the clinical symptoms and laboratory parameters in patients hospitalised with LNB. Additionally, we assessed independent predictors of positive anti-Borrelia antibody index.

**Methods** We retrospectively evaluated the data of patients hospitalized with LNB (possible, probable and definite). As probable LNB we considered patients with negative anti-*Borrelia* antibody index. No CSF analysis was performed in patients with possible LNB. Patient files were used to obtain demographic, anamnestic, clinical and laboratory data. Basic descriptive statistics were used in the first step followed by univariate and multivariate analysis using logistic regression.

**Results** In total, 56 patients with the diagnosis of LNB have been included in the analysis: definite (41), probable (9), possible (6). 23 patients (38%) were younger than 18 years, 46% were women. A lymphocytic pleocytosis was found in all but one patient with CSF analysis, but meningeal signs were positive only in 16% of patients. The most common clinical manifestations were facial nerve palsy (54%), headache (54%) and radicular pain (38%). 32% of patients had a history of Erythema migrans and 48% reported a tick bite. The production of anti-*Borrelia* antibodies in CSF was confirmed in 82% of patients. In a multivariate analysis, positive anti-*Borrelia* antibody index has been positively associated only with the facial nerve palsy (aOR 7.3; 95% CI 1.2-44.9; P = 0.032).

**Conclusion** CSF analysis is essential for reliable diagnosis of neuroborreliosis. Alternative diagnostic markers to anti-*Borrelia* antibody index are needed in patients with early LNB

P6 **Does treatment with corticosteroids influence the outcome of peripheral facial palsy in patients with confirmed Lyme borreliosis?**

Tereza Rojko1, Petra Bogovič1, Stanka Lotrič-Furlan1, Katarina Ogrinc1, Maša Velušček1, Eva Ružić-Sabljić1, Tjaša Cerar Kišek2, Franc Strle1

1Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia, 2Institute for Microbiology and Immunology, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

Objectives: To evaluate the outcome of peripheral facial palsy (PFP) in patients with confirmed Lyme borreliosis (LB) treated with corticosteroids as Bell's palsy before the diagnosis of LB was established and treatment with antibiotics was started.

Methods: Adult patients, who presented with PFP at our department in the period 2006–2017 and in whom diagnosis of LB was confirmed, were included in the study. LB was confirmed by the presence of at least one of four criteria: 1) presence or reliable history of recent erythema migrans; 2) intrathecal production of borrelial IgM and/or IgG antibodies; 3) isolation of *Borrelia burgdorferi* s.l. from CSF; 4) seroconversion to borrelial antigens within two months after the first examination. The evaluation of outcome was performed at control examinations 3, 6 and 12 months after the presentation.

Results: 102 patients (66 men and 36 women; median age 59; IQR 48-56) qualified for the study. Before the diagnosis of LB was confirmed and antibiotic treatment was started 19/102 (18.6%) patients were treated with corticosteroids (all with methylprednisolone, different dosages). Complete clinical remission of PFP at 3, 6 and 12 months after the first examination was achieved in 10/19 (52.6%) vs. 48/81 (59.3%) (p=0.391), 12/19 (63.2%) vs. 59/82 (72%) (p=0.310) and 13/18 (72.2%) vs. 67/79 (84.8%) (p=0.175) patients treated with corticosteroids vs. patients without corticosteroid treatment, respectively. In none of the patients other manifestations of LB developed during follow-up.

Conclusion: In the present study the outcome of PFP in the subset of 19 patients with confirmed LB, who were treated with corticosteroids for "Bell´s palsy" before the diagnosis of LB was established and antibiotics were prescribed, was not statistically significantly worse than the outcome in patients with confirmed LB without prior corticosteroid treatment. Furthermore, in none of the patients other manifestations of LB developed during follow-up.

P7 **A severe case of Lyme neuroborreliosis presenting with myelitis and complete tetraplegia**

Fredrikke Christie Knudtzen1, Anna Christine Nilsson2, Morten Blaabjerg3, Sigurdur Skarphedinsson1

1 Clinical centre for Emerging and Vector-borne Infections, Department of Infectious Diseases, Odense University Hospital, Denmark., 2 Department of Clinical Immunology, Odense University Hospital, Denmark., 3 Department of Neurology, Odense University Hospital, Denmark.

Lyme neuroborreliosis (LNB) typically presents with radiculitis, facial nerve palsy and lymphocytic meningitis. Severe cases of myelitis are rare.

**Case** A 40-year-old woman with paranoid schizophrenia presented at the Emergency Department with a two-week history of radiating pain and paresis of the right lower extremity (LE). She was afebrile, conscious and fully oriented. Cerebrospinal MRI showed no nerve-root compression. A functional disorder was suspected but due to progression in LE paresis, a lumbar puncture was performed showing lymphocytic pleocytosis and elevated protein. Intravenous ceftriaxone 2g qd was initiated on suspicion of LNB. Intrathecal production of *Borrelia* IgM/IgG antibodies confirmed the diagnosis.

Further symptom progression occurred with bilateral abducens and peripheral facial paralysis, complete paralysis of LEs and paresis in upper extremities. Despite three weeks of ceftriaxone and three-day high-dose prednisone, complete tetraplegia manifested requiring ventilator therapy. An additional 14 days of doxycycline was administered, testing for other tick-borne pathogens was negative.

The patient regained functions of upper extremities, and was decannulated after 31 days. Due to disease severity and slow recovery, the LNB diagnosis was questioned, and a broad screening for other diseases was initiated. Reported positive anti-*N*-methyl-D-aspartate receptor (anti-NMDAR) antibodies that were later deemed false positive complicated the course of admittance.

The patient was discharged to neurorehabilitation after 91 days. At follow-up one year later, she had regained upper body strength and sensory function in both LE.

**Discussion** We present a case of LNB with a rare manifestation of acute myelitis. Even though the patient fulfilled the criteria for definite LNB, extensive examinations were done to rule out other causes, resulting in false-positive anti-NMDAR results.

This case emphasizes that interpretation of test results and treatment should be based on each patient's clinical picture. It raises awareness of the risk of possible false-positive anti-NMDAR results in patients with LNB.

P8 **Can infection with Borrelia burgdorferi lead to neuronal loss or dysfunction in the brain?**

Adam Garkowski, Joanna Zajkowska, Urszula Łebkowska

Department of Radiology, Medical University of Białystok, Poland, Department of Infectious Diseases and Neuroinfections, Medical University of Białystok, Poland

**Introduction** There have been many research of investigating different central nervous system diseases and disorders using 1H-magnetic resonance spectroscopy (1H-MRS). The data obtained using this technique from **in vivo** tissue are unique, because they provide information about metabolic alterations. 1H-MRS may reveal changes while conventional magnetic resonance imaging (MRI) fails to detect any abnormality. We hypothesized that since *B. burgdorferi* causes systemic inflammation and infects the brain, it may lead to alterations in cerebral metabolism, as measured by 1H-MRS. We report results of a 1H-MRS study in 26 patients with early Lyme neuroborreliosis (LNB). The purpose of our study was to determine whether 1H-MRS could detect brain metabolite alterations in patients with early LNB in normal-appearing brain tissue on the conventional MRI. To the best of our knowledge, our study represents the largest series of patients with LNB investigated with 1H-MRS.

**Methods** Twenty-six patients diagnosed with early LNB and twenty-six healthy volunteers as a control group have been involved in the study. All of them underwent routine MRI protocol using 3.0 T MRI scanner. 1H-MRS examinations were performed with repetition time (TR) = 2000 ms, and echo time (TE) = 135 ms. Single voxels were positioned in the anterior and posterior part of the right and left frontal lobe.

**Results** We found a statistically significant decrease of the N-acetylaspartate/creatine ratio within the anterior part of the right and left frontal lobe ($p=\leq 0.001$ and $p=0.001$ respectively) and in the posterior part of the right and left frontal lobe ($p=\leq 0.001$ and 0.031) in the patients with LNB.

**Conclusion** A significant reduction in NAA/Cr ratio in comparison with the controls suggests the presence of diffuse neuronal loss or dysfunction in patients with early LNB.

P9 **Perceptions, representations and experiences of patients presenting nonspecific symptoms in a context of suspicion of Lyme borreliosis.**

Aude Barquin 1, K. Diallo 2, Olivier Patey 2, Alice Raffetin 2

1Department of General Practice, University Paris V René Descartes, 15 rue de l'Ecole de Médecine, 75006 Paris. 2Department of Infectious Diseases, CH Villeneuve Saint Georges, 40 allée de la Source, 94190 Villeneuve-Saint-Georges.

**Introduction** Lyme borreliosis (LB) mainly affects skin, joints, central/peripheral nervous system, and heart. Some subjective symptoms may also be present at all the stages (asthenia/polyalgia/cognitive complaints) and may persist several months after treatment (post-treatment Lyme disease syndrome). Nonspecific symptoms without any objective manifestation of LB are sometimes attributed by patients to a possible tick-bite. The aim of our study was to describe perceptions, representations and experiences of these patients of their disease and care paths.

**Materials/Methods** We performed a qualitative study (interviews) based on grounded theory to identify themes and to conceptualize a theory, respecting the COREQ items. Interviewed patients presented varied profiles (age, sex, high/low endemic area, positive/negative serology, taking part in a patient association or not) to obtain an illustrative sample of patients, from October 2017 to May 2018.

**Results** Twelve patients were interviewed**.** Data were saturated for the twelfth patient. 293 codes were identified, then classified in 8 themes that were not previously defined: (1)Disabling nonspecific symptoms, the first being pain; (2) A broken dialogue between patients and doctors, save their general practitioner but who remained overtaken; (3) Long and difficult care paths for the majority of the patients; (4) A representation of the disease as vicious, unknown and dangerous; (5) A break with the previous state of health, that required a reorganization of their way of life; (6) Multiple feelings, the firsts being fear and incomprehension; (7)The self-management of their disease; (8) The strong expression of a desire for change, with better care and recognition of their disease.

**Conclusion** Improving the management of patients with nonspecific symptoms associated or not with LB appears necessary even if it is not within the competence of the infectious disease physician. A multidisciplinary care organization could achieve a more precise diagnosis, including mostly differential diagnoses, and patient-centered medical support.

P10 **Lyme borreliosis or not Lyme borreliosis? A Multidisciplinary Approach Achieves An Accurate Diagnosis and Improves Patient-Centered Medical Support**

Alice Raffetin 1, Agathe Bounhiol 1, Anna Belkacem 1, K. Diallo 1, Julien Schemoul 2, S. Emilie 2, Giulia Paoletti 3, R. Jouenne 4, L. Lim 5, Corinne Canu 6, Sylvie Le Berre 6, Eric Meinadier 7, F. Medina 1, Pauline Caraux-Paz 1, and Olivier Patey 1

1Department of Infectious Diseases, 2Department of Rheumatology, 3Department of Psychiatry, 4Department of Internal Medicine, 5Department of Neurology, 6Department of Algology, 7Department of Physical Rehabilitation, All in Villeneuve-Saint-Georges Multidisciplinary Lyme Borreliosis Center, Centre Hospitalier Lucie et Raymond Aubrac, 40, allée de la Source, 94190 Villeneuve-Saint-Georges, France

**Background:** Because many patients suspected of having Lyme borreliosis (LB) experience **diagnosis wandering and difficult care paths,** we started the **Villeneuve-Saint-Georges Multidisciplinary Lyme borreliosis Center (VSG-MLC)** (joint endeavor of the departments of infectious diseases, internal medicine, rheumatology, neurology, algology, dermatology, psychiatry, microbiology, physical rehabilitation) in suburban Paris, France.

**Materials/methods:** We retrospectively analyzed the characteristics of all adults consulting at VSG-MLC mainly referred by their GPs (dedicated phone line), and their care paths at VSG-MLC (January 2018-February 2019). All the cases were discussed in a **multidisciplinary consultation meeting.** Patients with LB-linked symptoms were classified according to EUCALB/ESGBOR guidelines: **confirmed LB**; **possible LB** (tick exposure and/or prior erythema migrans, evocative clinical signs and marked clinical improvement after 28days of antibiotics); **post-treatment Lyme disease syndrome (PTLDS)** (asthenia/polyalgia/cognitive complaints) or **sequelae** (objective impairment), after proven LB treated as recommended; **monitoring after a tick-bite**; and **cured patients**.

**Results:** 191 patients consulted: 108/191(56.5%) were followed in external consultations, 51/191(26.7%) hospitalized and/or 62/191(32.5%) underwent a 1-day hospitalization stay, with 407 follow-up consultations. Seventy-one (37.2%) patients had LB-associated symptoms: 30/191(15.7%) confirmed LB, 11/191(5.8%) possible LB, 11/191(5.8%) PTLDS, 6/191(3.1%) sequelae, 5/191(2.6%) post-tick–bite monitoring, 8/191(4.2%) cured LB. Associated or differential diagnoses were found for 157/191(82.2%) patients: other infectious diseases (21/191;11%), rheumatological/autoimmune diseases (42/191;22%), bodily distress syndrome (20/191;10.5%), neurological diseases (18/191;9.4%), psychiatric diseases (16/191;8.4%), previous long-term antimicrobial side-effects (16/191;8.4%), deficiencies/metabolic diseases (12/191;6.3%), others (12/191;6.3%). Twenty-eight patients (14.7%) had no diagnosis. **All patients received patient-centered care in the adapted sector to treat any disease or symptoms**—even without a definitive diagnosis—especially pain.

**Conclusions:** As previously found in high endemic areas, **~15% of suspected cases had confirmed LB**. The **multiplicity of associated or differential diagnoses** highlights the complexity of diagnosing LB, without disregarding other diagnoses, and vice versa. **A multidisciplinary care organization achieves a more precise diagnosis and patient-centered medical support.**

P11 **Human tick-borne encephalitis and correlation with rodents and big game in Croatia in the period from 2006 to 2017**

Linda Bjedov 1, Kristijan Tomljanović 1, Marko Vucelja 1, Josip Margaletić 1, Nataša Cetinić Balent 2, Oktavija Đaković Rode 2, 3

1 Department of Forest Protection and Wildlife Management, University of Zagreb Faculty of Forestry, Zagreb, Croatia, 2 University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb, Croatia, 3 University of Zagreb School of Dental Medicine, Zagreb, Croatia

Tick-borne encephalitis (TBE) is considered endemic in continental parts of Croatia. Many factors, such as ecological, climatic and social factors, can influence the epidemiology of TBE but are still not sufficiently understood. TBE virus circulates between ticks and their mammal hosts, mostly rodents and deer. There are not enough studies in Croatia that research the correlation between tick hosts and human cases of TBE. The aim of this study was to compare and correlate the numbers of TBE acute patients with tick mammal hosts. For this analysis we used available forest rodent and big game (roe deer, red deer and wild boar) abundance data for the time period from 2006 to 2017. Rodent data is presented as annual rodent damage per hectare, reported annually from forestry offices in continental Croatia. Big game data for each mentioned species is presented as annual number of harvested individuals. In the 12-year period a total of 14154 sera were tested for IgM and IgG anti-TBE. Seropositive were 759 patients, out of whom 588 had acute TBE. For comparison of variables, Pearson's correlation coefficient (r) was used. There was no correlation found between acute patients and rodent abundance (r= -0.04) as well as wild boar abundance (R= -0.05). Roe deer abundance and acute patient data showed slightly positive correlation (R= 0.22) which was similar but higher for red deer (R= 0.37). In literature, a positive correlation is also described between deer species and tick abundance in forests, which correlated with our findings indicating higher risk for humans in years of higher deer abundance. Our results contribute to other findings described in the literature regarding the relation between deer species, ticks and TBE cases.

P12 **The molecular – epidemiological characteristic of tick-borne encephalitis natural foci and other tick-borne infections in the Baikal region of Russia**

Dmitriy O. Kiselev1, Stepanenko L.A.1, Dzhioev Yu.P.1, Badmaev A.A.2, Galsanov B.Ch.2, Khanhareyev S.S.2, Zlobin V.I.1

1 Scientific research institute of Biomedical technologies of Irkutsk State Medical University, Irkutsk, Russia, 2Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing of The Republic of Buryatia, Ulan-Ude, Russia

The Baikal region of Russia is distinguished by the greatest genetic diversity of TBEV, an abundance of natural foci of combined tick-borne infections. Using the methods of molecular biology, the study of natural foci of tick-borne encephalitis, borreliosis, ehrlichiosis and anaplasmosis in the territory of the Baikal region (3 districts of the Irkutsk region and 7 districts of the Republic of Buryatia) was conducted. A current information on infection in ticks (Ixodes persulcatus) by TBEV, *Borrelia*, *Ehrlichia* and *Anaplasma* in the study areas was obtained. A different degree of tick infestation by these pathogens was noted on the territory of all surveyed areas. In some areas of the Republic of Buryatia, a high degree TBEV-infection (up to 10%), as well as *Borrelia* (up to 90%), *Anaplasma* (up to 45%) and *Ehrlichia* (up to 40%) was noted. Used TBEV typing method directly in mite homogenates and thus excluding laboratory manipulations, which in some cases led to virus variability as a result of penetration into sensitive systems. For this purpose, genotype-specific primers have been developed that are complementary to specific regions of the TBEV genome of the three main genotypes (Far-Eastern, Western and Siberian) and the group of strains 886-84 (“Baikal genotype”). Using this method in this sample of ticks, TBEV circulation of the Siberian and Far Eastern genotypes was established. The information on cases of ticks mixed infection by TBEV and other tick-borne pathogens was also obtained. The highest ticks mixed infection was observed in the northern areas of the Republic of Buryatia (Barguzinsky and Kurumkansky districts). The species diversity of pathogens of genus *Borrelia* on the territory of the Baikal region was studied using the Real-Time PCR method. In the homogenates of collected ticks pathogens of species *B. garinii*, *B. afzelii* and *B. miyamotoi* were found.

P13 **Cytokine response in vaccine breakthrough patients with tick-borne encephalitis**

Miša Pavletič A, Misa Korva A, Nataša Knap A, Petra Bogovič B, Mirijam Nahtigal Klevišar B, Tomaž Vovko B, Janez Tomažič B, Stanka Lotrič-Furlan B, Franc Strle B, Tatjana Avšič Županc A

A Laboratory for diagnostic of Zoonoses and WHO Center, Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, B Department of Infectious Diseases, University Medical Center Ljubljana, Ljubljana, Slovenia

Tick-borne encephalitis (TBE) is one of the most important viral infections of the central nervous system in Europe. Currently, no effective antiviral therapy is available, patient care is mainly supportive. The most effective protection against TBE is vaccination which successfully triggers the formation of neutralizing antibodies in 98-99.5% of vaccinated individuals, however, some vaccine breakthroughs (VBTs) have been described. Although a few studies investigating the characteristics of antibody response in VBT patients have been performed, there is no information on their immunological features available. Thus, the aim of our study was to investigate cytokine responses in VBT patients. Levels of 35 cytokines and chemokines were measured in serum and CSF samples obtained during meningoencephalitic phase of illness in 41 VBT patients and 78 age and gender matched nonvaccinated patients with TBE, as well as in serum of 42 healthy controls. The measurements were performed using multiplex immunoassay on Magpix (Luminex) instrument. In patients with TBE, serum levels of several cytokines and chemokines differed from those found in healthy controls but there were also some differences within the TBE group. In comparison to nonvaccinated patients with TBE, VBT patients had significantly higher levels of VEGF, IL-2 and IL-15 in serum and CSF but lower serum concentrations of IL-21 and BCA-1/CXCL-13, while elevated serum levels of IL-1β and IL-23 were detected only in nonvaccinated TBE patients. Our results suggest that although VBT and nonvaccinated TBE patients share some immunological features they certainly differ in the extent and orientation of proinflammatory immune responses.

P14 **Active surveillance for the incidence of tick-borne encephalitis in Poland on non endemic areas**

Joanna Zajkowska, Elzbieta Waluk, Justyna Dunaj, Renata Swierzbinska, Sławomir Pancewicz

Department of Infectious Diseases and Neuroinfections Medical University in Białystok

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.

**Aim** 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.

**Methods** 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:

**Results** In 2018 were reported less cases of TBE than in 2017. During ongoing the study, cases out of official records, new cases of TBE are confirmed. Some positive samples were from areas of low incidence, and some in quite new areas.

**Cocnclusions** Actually results suggestthat 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.

P15 **Factors associated with levels of IgG antibodies to tick-borne encephalitis virus during early meningoencephalitic phase of tick-borne encephalitis**

Petra Bogovič, 1 Lara Lusa, 2, 3 Stanka Lotrič-Furlan, 1 Miša Korva, 4 Miša Pavletič, 4 Tatjana Avšič-Županc, 4 and Franc Strle1

1 Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia, 2 Institute for Biostatistics and Medical Informatics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, 3 Department of Mathematics, Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Koper, Slovenia, 4 Institute for Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia

**Background** Information on parameters associated with levels of IgG antibodies to tick-borne encephalitis virus (TBEV) in serum or CSF of patients with TBE is limited.

**Methods** The association between 13 pre-defined clinical and laboratory parameters (patient age, sex, presence of underlying illnesses, previous vaccination against TBE, duration of illness, monophasic course of illness, blood leukocyte count, serum C-reactive protein level, CSF leukocyte count, CSF protein concentration, concomitant Lyme neuroborreliosis, presence of serum IgG antibodies to *Borrelia burgdorferi* sensu lato, and the severity of acute illness) was evaluated in the initial serum (717) and CSF (81) specimens, obtained from adult patients diagnosed with TBE at a single medical centre in the period 2007–2012.Severity of illness was defined clinically as mild (patients having meningitis) or severe (meningoencephalitis or meningoencephalomyelitis) and quantitatively using a severity score.

**Results** Multivariable analyses disclosed that male sex (estimated coefficient, EC 0.86,95% CI 0.74–0.99; *P*=0.051), previous vaccination against TBE (EC 12.51, 95% CI 7.61–17.41; *P*<0.001), duration of illness (EC 1.41, 95% CI 1.16–1.67; *P*<0.001), serum C-reactive protein level (EC 1.31, 95% CI 1.07–1.55); *P*=0.005), CSF leukocyte count (EC 0.84, 95% CI 0.71–0.97; *P*<0,001), and severity of acute illness based on clinical presentation as well as on the severity score (EC 0.72, 95% CI 0.65–0.78; *P*<0.001) were associated with levels of serum IgG antibodies to TBEV. The only covariate associated with levels of CSF antibodies to TBEV was severity of illness (EC 0.67, 95% CI 0.51–0.83; *P*=0.001).

**Conclusions** During early meningoencephalitic phase of TBE levels of IgG antibodies to TBEV in serum are associated with male sex, previous vaccination against TBE, duration of illness, serum C-reactive protein level, CSF leukocyte count, and severity of acute illness, while levels of CSF antibodies to TBEV is associated with severity of illness.

P16 **First Human Case of Tick-Borne Encephalitis on the Island of Funen, Denmark**

Nanna Skaarup Andersen and Sigurdur Skarphédinsson

Clinical Centre for Emerging and Vectorborne Infections, Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark and, Clinical Centre for Emerging and Vectorborne Infections, Department of Infectious Diseases, Odense University Hospital, Odense, Denmark

In Denmark, Tick-Borne Encephalitis (TBE) has been known to exist only on the island of Bornholm since the 1950s. In 2009 two new cases from North Zealand were identified. Until 2018 no new cases were identified outside Bornholm, but serological studies using roe deer as sentinels suggest that TBE-complex viruses do exist all over Denmark and that foci have been expanding since the turn of the millennia. We present the first human case of TBE from Fåborg on the island of Funen, an area suggested as possible foci using roe deer sentinels.

Case: In early October 2018 a 39-year-old woman develops a transient febrile illness. The symptoms regress during the following week, but were followed by the onset of headache, photophobia, nausea and vomiting. In late October the patient seeks medical advice due to constant severe headache and reemergence of fever (39.4 °C). No history of tick bite. No recent travel history, including travel to Bornholm, Sweden or Eastern Europe. Never vaccinated against TBE, Japanese encephalitis or Yellow fever. Objective examination reveals neck-pain, but no stiffness. Normal neurological examination. Normal CT-cerebrum. CSF: Leucocytes 72 x 10E6/L, of these 67 x 10E/6L monocytes. Blood: Leucocytes 14.7 x 10E9/L. The tentative diagnosis was viral encephalitis. Microbiology findings; CSF: negative culture, negative PCR for HSV-1, HSV-2, VZV and enterovirus. Negative inthrathecal test for *Borrelia burgdorferi* and negative CXCL-13 < 10. Negative blood culture.

Due to the biphasic course TBE is suspected and supported by the finding of anti-IgG and anti-IgM TBE antibodies by ELISA test. Confirmatory TBE-virus neutralization test showed a titer of 640, verifying the TBE diagnosis.

Conclusion: Our case identifies a new TBE risk area in Denmark and supports that roe deer used as sentinels can identify new TBE risk areas, and monitor the changing distribution of TBE.

P17 **Significance and value of laboratory data in tick-borne encephalitis virus infection: a north-eastern Italy experience**

Nicole Barp, Mariachiara Di Nuzzo, Alessandro Trentini*, Martina Maritati, Valeria Mondardini**, Ermenegildo Francavilla**, Carlo Contini

Dept Medical Sciences, Section of Infectious Diseases, University of Ferrara, Italy; Dept Biomedical Sciences, Section of Biochemistry, Molecular Biology and Medical Genetics, University of Ferrara, Italy*; Infectious Diseases, Medical Hospital "San Martino", Ulss1 Dolomiti, Belluno, Italy**

**Introduction** Unlike TBEVi clinical manifestations, laboratory data (LD) are rarely described. Abnormal LD in course of TBEVi could help clinicians in diagnosis and follow-up timing.

**Methods** We retrospectively analyzed (June 2000-April 2019) 147 TBEVi cases from Infectious Disease Unit of Belluno, Italy. LD were recorded and statistically analyzed, particularly abnormal LD and the phase of TBEVi (1st or 2nd) relationship and abnormal LD and sequelae relationship.

**Results** We recorded 112 males and 36 females, mean age 53 years, with a monophasic (29%) or biphasic course (71%). 95% presented abnormal complete blood count: neutrophilia (69%), monocytosis, lymphocytopenia (52%, each), leukocytosis (51%), thrombocytopenia (26%), leukopenia (24%), neutropenia (15%), basophilia (10%), lymphocytosis (8%), thrombocytosis and monocytopenia (2%, each). Thrombocytopenia, neutropenia and lymphocytosis were associated with the 1st phase ($p<0.01$); monocytosis and lymphocytopenia with the 2nd one ($p<0.05$). High C-reaction protein (CRP) and erythrocyte sedimentation rate (ERS) levels (50%, each) were associated with the 2nd phase ($p<0.05$). Moreover, 81% had abnormal liver tests: hyperbilirubinemia (36%), high levels of gamma-glutamyltransferase (GGT, 23%), alanine aminotransferase (ALT, 21%) and aspartate aminotransferase (AST, 14%). 45% patients had electrolyte disorders: hyponatremia (32%), hypochloremia (31%) and hypokalemia (18%). We observed high levels of fibrinogen (41%), creatine phosphokinase (CPK, 29%), lactate dehydrogenase (LDH, 12%) and amylase (10%). Abnormal liver tests, electrolyte disorders, abnormal fibrinogen, CPK, LDH and amylase did not seem to be associated with a specific phase ($p>0.05$). All LD results were not associated with sequelae ($p>0.05$).

**Conclusion** Thrombocytopenia, neutropenia and lymphocytosis seem to characterize the 1st phase; monocytosis and lymphocytopenia, high RCP and ERS levels, the 2nd one; LD results are not predictive of sequelae. In contrast with other previous studies, we observed a higher prevalence of abnormal liver tests and electrolyte disorders. Abnormal levels of fibrinogen, CPK, LDH and amylase have been never described previously.

P18 **Ticks and tick-borne pathogens: emerging health threats on the Italian side of the Western Alps**

Aitor Garcia-Vozmediano1, Aleksandra Iwona Krawczyk2, Hein Sprong2, Luca Rossi1, Elisa Ramassa3 and Laura Tomassone1

1 Department of Veterinary Sciences, University of Turin, 10095 Grugliasco, Italy, 2 National Institute of Public Health and Environment (RIVM), 3720 BA Bilthoven, The Netherlands., 3 Ente di Gestione delle Aree Protette delle Alpi Cozie, 10050 Salbertrand, Italy

The geographical expansion of ticks and tick-borne diseases (TBD) in Europe is a fact, favored by climate and ecosystem changes. In Northwestern Italy, ticks are an emerging concern, in particular *Ixodes ricinus* – the main vector of TBD in Europe. Tick-bites and human cases of Lyme borreliosis are on the rise in the Alpine region, even though they are probably under-diagnosed and underreported. Since 2016, we are carrying out monitoring activities on a local scale with an integrated approach, with surveillance: i) on ticks from the environment, domestic animals and wildlife; ii) on human tick bites and disease cases, in close collaboration with physicians. Different stakeholders are being involved in our activities: forestry workers, hunters, farmers, hikers. Our results demonstrate the presence of *I. ricinus* over 1800m a.s.l., in mountainous areas where this tick was absent in the past century. In addition, molecular analyses disclosed infections by bacteria belonging to the *Borrelia burgdorferi* sensu lato complex (*B. afzelii, B. garinii, B. valaisiana* and *B. burgdorferi* sensu stricto; 15.5% of tested nymphs), Spotted Fever Group rickettsiae (*Rickettsia helvetica* and *R. monacensis*; 20.7%), followed by *Anaplasma phagocytophilum* (1.9%), *Candidatus* Neoehrlichia mikurensis (0.5%), and the relapsing fever spirochete *Borrelia miyamotoi* (0.5%). *Rickettsia slovaca* – causative agent of the 'tick-borne lymphadenopathy', was also detected in *Dermacentor marginatus*, the other tick species collected by dragging and on animals. Given the low awareness among the population and the complexity of TBD epidemiology, we believe that the integrated approach adopted may help in mitigating TBD impact on public health and provide scientific evidence for surveillance and prevention, and to inform decision makers.

P19 **Diversity and prevalence of tick-borne pathogens in engorged Ixodes ricinus ticks collected from humans in Romania**

Zsuzsa Kalmár*1, Ioana Adriana Matei1, 2; Mirabela Oana Dumitrache1; Gianluca D'Amico1; Mihaela Lupșe3; Andrei Daniel Mihalca1

1Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania., 2Department of Microbiology, Immunology and Epidemiology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania., 3Department of Infectious Diseases, University of Medicine and Pharmacy, Cluj-Napoca, Romania

*Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur, 3-5, 400372 Cluj-Napoca, Romania. Tel: +40722834993, email address: zsuzsa.kalmar@usamvcluj.ro

Ticks are the medically important vectors of infectious diseases, capable of transmitting pathogens to humans and animals and are competent vectors for a large number of medically important pathogens. Tick-borne diseases play a major role in the public health and have been increasing the public health risk during the last century, being reported across Europe, still affecting millions of people each day. The aim of our study was to provide epidemiologic data regarding the presence of tick-borne pathogens in ticks feeding on humans in Romania.

We screened 525 *Ixodes ricinus* ticks (84 females, 420 nymphs, 21 larvae) collected from humans during the same season in two consecutive years in north-western part of Romania. Conventional and quantitative PCR was used to detect specific genes of each pathogen. For identifying the infectious agents, positive samples were further analysed by conventional PCR and were sequenced. The detected agents were *Borrelia* spp. (14.1%), *Rickettsia* spp. (7.4%), *Neoehrlichia mikurensis* (6.0%), *Anaplasma phagocytophilum*. (5.0%). Overall, 14.3% of the larvae, 23.3% of the nymphs and 31% of the females harboured at least one zoonotic pathogen. From these, 21.3% were co-infections with different species of tick-borne pathogens. No significant differences of pathogens prevalence between the two years study period were observed.

Our study is the first report of multiple tick-borne infections in ticks attached to humans in Romania. The potential improvement of screening the distribution of tick-borne pathogens in ticks collected from humans may provide new insights in understanding the complex ecology of tick-borne diseases, and enlighten about the infection prevalence at local, regional and national level.

P20 **Ixodes-borne pathogens in the Akfadou forest in Algeria**

Pierre H. Boyer (1), Mohand Oukaci Brahami (2), Dina Daaboul (1), Émilie Talagrand-Reboul (1), Cathy Barthel (1), Benoît Jaulhac (1-3), Mohamed Boukhemza (2), Nathalie Boulanger (1-3)

(1)EA 7290: Early Bacterial Virulence: Borrelia Group, CHRU Strasbourg, Fédération de Médecine Translationnelle de Strasbourg, F-67000 Strasbourg, France., (2)Laboratory of Ecology and Biology of Terrestrial Ecosystems, Faculty of Biological Sciences and Agronomic Sciences. University Mouloud Mammeri. Tizi-Ouzou, Algeria., (3)French National Reference Center for Borrelia, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

There are few data on the vector epidemiology of *Ixodes*-borne pathogens in Algeria. We therefore investigated the presence of three *Ixodes*-borne pathogens in the unexplored forest of Akfadou in the region of Tizi-Ousou in Algeria.

Of the 1,000 adults *Ixodes ricinus* ticks, collected on cattle in three separate sites during a two-year period (2015-2016), 450 were selected for molecular analysis. *Borrelia burgdorferi* sensu lato (Bbsl), *Anaplasma phagocytophilum* (Ap), *Candidatus* Neoehrlichia mirkurensis (CNm) and relapsing fever *Borrelia* (RFB) were searched using real-time PCR assays targeting specific gene for the microorganism of interest. Subsequent sequencing was used for the identification of the *B. burgdorferi* sl and RFB genomospecies.

On 450 ticks, 107 were positive for Bbsl (23.8%), 10 for Ap (2.2%), 2 CNm and one RF *Borrelia*. One tick carried both Bbsl and Ap. The sequencing of the *flaB* gene revealed that 105 of the Bbsl had on average homology of 98% with the published sequences of *B. lusitaniae*. One *B. valaisiana* and one *Bbss* were also identified. Interestingly, the RFB clusters along with the other hard-tick RFB but needs to be better characterized.

The region of Akfadou near Tizi-Ousou seems to be a region of endemicity for a *B. lusitaniae* particular strain, which needs to be better characterized by future analysis. Further studies are needed to investigate if this *Borrelia*-strain can cause Lyme disease. This study described for the first time *B. valaisiana* in Algeria and more surprisingly CNm in this region.

The prevalence of ticks carrying Ap is as prevalent as it is observed in Europe, where clinical cases are observed each year in several EU countries.

In addition, patients presenting fever upon a tick bite should be investigated to evaluate the involvement of Ap and CNm in this region of Africa.

P21 **Diagnostic of Lyme borreliosis: comparison of serological assays in twelve clinical laboratories in Northern Europe**

Malin Lager 1, 2, Ram B. Dessau 3, Peter Wilhelmsson 1, 2, Dag Nyman 4, Guro F. Jensen 5, Andreas Matussek 1, 6, 7, Per-Eric Lindgren 1, 2, Anna J. Henningsson 1, 2 The ScandTick Biobank Study Group

1 Clinical Microbiology, Laboratory Medicine, Region Jönköping County, Sweden, 2 Medical Microbiology, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden, 3 Department of Clinical Microbiology, Slagelse Hospital, Slagelse, Denmark, 4 The Åland Group for Borrelia Research, Mariehamn, Åland, Finland., 5 Department of Medical Microbiology, Sørlandet Hospital Trust, Kristiansand, Norway, 6 Karolinska University Laboratory, Stockholm, Sweden, 7 Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden.

Lyme borreliosis (LB) is the most common tick-transmitted disease in Europe. The diagnosis of LB is based on the patient´s medical history, clinical signs and symptoms in combination with laboratory analyses. In clinical practice, serological detection of *Borrelia* specific antibodies by enzyme-linked-immunosorbent assay (ELISA) has been widely used, sometimes supplemented by immunoblot in order to increase the specificity. Current ELISA methods have high analytical sensitivity and specificity, besides being inexpensive and easy to perform. However, there are some limitations in clinical interpretation due to biological aspects that need to be taken into consideration, like the delay in antibody response in early LNB, possible IgM cross-reactivity with other pathogens and persistence of antibodies after the infection. The objective of this study was to evaluate the diagnostic sensitivities and specificities of different serological ELISA methods (IgM and IgG) that are currently in use for LB diagnosis in clinical laboratories in Northern Europe. The methods were evaluated by using a large and well characterized panel of patients and control sera. The panel consisted of 195 serum samples from well characterized patients under investigation for clinically suspected LB including patients with active Lyme neuroborreliosis, Lyme arthritis, acrodermatitis chronica atrophicans, erythema migrans or other diseases (non-LB controls). A total of 201serum samples from blood donors were also included. The panel (n=396) was sent to 12 clinical laboratories (using five different ELISA methods) as blinded and the laboratories were asked to analyse them according to their routine laboratory procedure. The results from the study demonstrated high concordance between the laboratories using the same type of diagnostic assay and lower concordance between laboratories using different types of diagnostic assays. Both the inter- and intra-assay comparison had more homogeneous results with high sensitivity, >90 % for IgG, compared to IgM which showed lower specificity and more heterogeneous results.

P22 **Performance of LightMix® Modular Borrelia spp. on samples from official proficiency panels**

Tjaša Cerar Kišek, Meta Kodre, Vesna Cvitković-Špik, Eva Ružić-Sabljić

Institute of Microbiology and Immunology, Faculty of Medicine Ljubljana, Slovenia

**Background** PCR enables direct detection of *Borrelia* spp. in different sample types, methods differ in target regions and assay formats. To increase sensitivity, reverse transcription and amplification of ribosomal RNA are included in run protocol of LightMix® Modular *Borrelia* spp (TIB MolBiol, Germany). The aim of the study was to asses performance of LightMix® Modular *Borrelia* spp. using well-defined samples.

**Materials and methods** To asses performance of LightMix® Modular *Borrelia* spp. (TIB MolBiol, Germany) we included 89 samples from official proficiency panels (INSTAND, Germany; QCMD, UK) and compared the results with routinely used method, *artus Borrelia* LC PCR Kit (Qiagen, Germany). Nucleic acid was isolated using Magna Pure Compact or Magna 24 System (Roche, Germany). According to INSTAND and QCMD, *B. burgdorferi* sensu lato and relapsing fever *Borrelia* were present in 69 and 6 samples, respectively.

**Results** Both tests were positive in 69/83 (83.1 %) and gave concordant results in 83/89 (93.3 %) of the samples. As a*rtus Borrelia* LC PCR Kit detected *B. burgdorferi* sensu lato in 67 samples and *B. miyamotoi* in 2 samples, LightMix® Modular *Borrelia* spp. additionally detected *B. burgdorferi* sensu lato and relapsing fever *borrrelia* (2 *B. miyamotoi*, 1 *B. turicatae*, 1 *B. duttoni*) in 2 and 4 samples, respectively. Median ct values of LightMix® Modular *Borrelia* spp were lower in comparison to *artus Borrelia* (25.89 vs 30.48), suggesting higher test sensitivity.

**Conclusion** LightMix® Modular *Borrelia* spp. is a rapid and sensitive assay. Test has a reasonable price and it can be combined with other modular tests.

P23 **Development of a novel easy to use protein microarray for Borrelia diagnostics**

Julia Schamberger, Stefan Ammer, Michael Mayer, Torsten Pirch, Angela Eser, Ingrid Albrecht-Walz, Friedhelm Struck, Karin Wochinz-Richter, Kerstin Wagner, Oliver Böcher, Erwin Soutschek

Mikrogen GmbH, Floriansbogen 2-4, 82061 Neuried

**Objective** Lyme Borreliosis, caused by five known species of the *Borrelia burgdorferi* sensu lato complex (*B. b.* sensu stricto, *B. garinii*, *B. afzelii*, *B. spielmanii*, *B. bavariensis*), is the most common tick-borne disease in Europe. In routine diagnostics patient samples are analyzed in a two-step process consisting of a screening test, e. g. ELISA, and a confirmation test, e. g. immunoblot. However, processing and evaluating immunoblots can be very time consuming. Therefore, we introduce an alternative confirmatory test system which combines the advantages of ELISA and Line Immunoblot techniques into one assay (r*ecom*Dot Borrelia).

**Methods** Recombinant *Borrelia* proteins covering all five pathogen species are spotted on nitrocellulose membrane and fixed in cavities of a 96 well plate. Proof of concept was shown by analyzing more than 600 sera including clinical samples, blood donor samples, cross-reacting sera and negative controls using a novel highly intuitive software.

**Results** The new *recom*Dot *Borrelia* test system shows a combined diagnostic IgG/IgM sensitivity of 100 % for the late disease manifestations Lyme-Arthritis and Acrodermatitis chronica atrophicans, 96% for neuroborreliosis and 88% for erythema migrans cases, including some erythema chronicum migrans cases. Specificity has been shown to be 100% comparing *recomDot* Borrelia result with sera negative analyzed in two other commercially available test systems.

**Conclusions** The *recom*Dot *Borrelia* has turned out to be a fast, easy-to-use and a more economical alternative to conventional methods confirming screening results. The *recom*Dot *Borrelia* is highly sensitive and specific for the detection of antibodies against *Borrelia burgdorferi* ssp. and can be easily processed and a fully automated.

P24 **Point-of-care detection of recent Borrelia infections using Sofia Lyme+ as Tier-1 assay**

Marlies van Lent, Ashlee King, Lauren Hedges, Rob Reed, Anja Garritsen

Innatoss Laboratories, Quidel Corporation

**Background** For Lyme testing, a two-tier strategy is used. Enzyme immunoassays (EIA) used as Tier-1 test detect either IgM or IgG antibodies against *Borrelia* spp. Sofia 2 Lyme+ fluorescent immunoassay (FIA) is a point-of-care test developed for rapid detection of both IgM and IgG antibodies against European *Borrelia* strains from a single sample. Testing in near-patient environments leads to faster diagnosis.

**Methods** Detection of recent *Borrelia* infections by means of seroconversion was compared between Sofia FIA and classic two-tier testing (IgG, IgM, and C6 EIA, followed by immunoblot). Longitudinal samples of subjects in 2 Dutch cohorts were tested: 1) outdoor workers in an annual Lyme screening program, seroconverting between consecutive measurements (n=25), and 2) study participants with a recent tick bite (tested after the bite and after 4 and 12 weeks), who seroconverted during the study (n=24).

**Results** In the annual Lyme screening group, 23 of 25 (92%) seroconversions detected by two-tier testing were confirmed by Sofia FIA. In comparison, when either IgG & IgM EIA's or the C6 EIA are used as Tier-1 tests, only 21 of 25 seroconversions (84%) could be identified. In the tick bite study group, 21 of 24 (88%) seroconversions were detected by Sofia FIA. While Tier-1 testing with IgG & IgM EIA's could also detect 21 of 24 seroconversions (88%), only 19 of 24 (79%) were identified using the C6 EIA.

**Conclusion** Sofia 2 Lyme+ FIA is a sensitive easy to use point-of-care assay for detection of *Borrelia* antibodies. When used as Tier-1 assay, Sofia FIA identifies a higher percentage of seroconversions than by testing with either IgG & IgM EIA or C6 EIA. For patients with a doubtful erythema migrans or non-specific complaints after tick bites, Sofia FIA provides general practitioners a fast diagnosis which may help resolve symptoms and prevent disease progression.

P25 **Sensitivity and Specificity of a Novel Tier 1 Lyme Assay in Europe**

L. Hedges, C. Charles, A. King, N. Lane, I. Blandy, R. Reed, Anja Garritsen, Marlies van Lent, J. Sun, R. Egan, J. McClure

Quidel Corporation, San Diego, United States and Innatoss Laboratories, Oss, Netherlands

In Europe, Lyme disease is primarily caused by *B. afzelii*, *B. garinii* and *B. burgdorferi* s.s. Quidel has developed the Sofia 2 Lyme+ FIA – a novel point-of-care Tier 1 Lyme lateral flow assay that qualitatively detects the presence of IgM and/or IgG antibody against various *Borrelia* strains that are present in Europe. Using a proprietary bi-directional assay format, the Sofia 2 Lyme+ FIA qualitatively reports differential Lyme IgM and Lyme IgG results from a single serum or plasma sample within 3 to 10 minutes.

Samples were collected in Europe from individuals suspected of having Lyme disease and/or were at high risk of Lyme disease due to their field of work. These samples were tested with the Sofia 2 Lyme+ FIA, and a predicate IgG and IgM EIA. First tier positive and equivocal results were tested on a predicate IgM and IgG Western Blot. Additionally, samples confirmed positive by western blot were collected that contained at least one positive band for the following strains: *B. afzelii*, *B. garinii*, *B. spielmanii*, and *B. bavariensis*. These samples were tested with Sofia 2 Lyme+ FIA and the % positivity was determined.

The sensitivity and specificity of the Sofia Lyme+ FIA IgM is 92.3% and 90.1%, respectively. The sensitivity and specificity of the Sofia 2 Lyme+ FIA IgG is 96.4% and 95.2%, respectively. Sofia 2 Lyme+ FIA performance is comparable to existing European Tier 1 and Tier 2 methods when tested with serum/plasma from patients that were suspected of Lyme disease in Europe. In addition, Sofia 2 Lyme+ FIA detects samples that represent each of the circulating strains of *Borrelia* in Europe. Sofia 2 Lyme+ FIA is an easy-to-use, rapid assay that can be used in a point-of-care setting, qualitatively reporting the presence of *Borrelia* IgM and IgG antibodies between 3 to 10 minutes.

P26 **VICTORY: Validation of cellular tests for Lyme borreliosis**

Freek R. van de Schoor, MD 1*; M.E. Baarsma, MD 2*; C.C. van den Wijngaard, PhD 3; L.A.B. Joosten, PhD 1; B.J. Kullberg, MD, PhD 1; J.W.R. Hovius, MD, PhD 2, #

Affliations 1. Radboudumc, Department of Internal Medicine and Radboud Center for Infectious Diseases, Nijmegen, the Netherlands 2. Amsterdam UMC, University of Amsterdam, Center for Experimental and Molecular Medicine, Amsterdam Institute of Infection and Immunology, Amsterdam, the Netherlands 3. National Institute for Public Health and the Environment (RIVM), Center of Infectious Disease Control, Bilthoven, the Netherlands. * Both authors contributed equally to this work. # Corresponding author: victory@amc.nl

**Introduction** The current cornerstone of diagnostics for Lyme borreliosis (LB) is serology, however, this has several shortcomings. Antibody production may be absent in the early phase of the disease, and once IgG-seroconversion has occurred, it can be difficult to distinguish between a past (cured or self-cleared) LB, and an active infection. It has been postulated that cellular tests for LB have both higher sensitivity earlier in the course of the disease, and are able to discriminate between a past and active infection. Several of such tests are already commercially available in Europe and patients present the test results in consultations with their physicians. Cellular LB tests lack thorough and independent validation, however.

**Methods** We present the design of a comprehensive validation study for several cellular tests for LB. Our study is a prospective two-gate case-control study. We are including patients who meet the European case definitions for either localized or disseminated LB. Four different cellular tests, either under development or commercially available, as well as two-tier serology is performed around the start of antibiotic treatment and after 6 and 12 weeks. We are also including healthy controls without current LB and controls with potentially cross-reactive conditions (infectious or auto-inflammatory), both seronegative and seropositive for LB.

**Results/Conclusion** This study started in May 2018 and inclusion is ongoing. Currently, we have collected 859 samples from 418 individuals. The findings of our study will help to better appreciate the utility of cellular tests in the diagnosis of Lyme borreliosis, and its possible use as a point-of-cure test.

P27 **The utility of Borrelia-specific T cells as diagnostic biomarkers of Lyme disease**

Amir Ameri1, Sambor Grygorczuk3, Liselotte Brix1, Kivin Jacobsen1, Yngvar Tveten2 and Dag Nyman4

1Immudex, Copenhagen, Denmark; 2Sykehuset Telemark, Skien, Norway; 3Medical University in Bialystok, Bialystok, Poland; 4Bimelix, Mariehamn, Åland

Most diagnostic tests for Lyme disease are based on detection of the antibody response against *Borrelia burgdorferi* sensu lato in blood. The diagnostic performance of the antibody tests is challenged by the facts that the antibody response may take weeks to appear and is detectable in the blood months to years after the active infection, thereby limiting early diagnosis and differentiation between patients with active disease and past cleared infection.

We investigated the potential of measuring *Borrelia*-specific T-cells in blood as a supplementary diagnostic marker for differentiation between active and past cleared infection. A *Borrelia* Dextramer assay was developed for direct measurement of *Borrelia*-specific T cells in blood by using flow cytometry. The assay is based on *Borrelia*-specific MHC Dextramers, which are fluorochrome-conjugated MHC multimers displaying T cell epitopes from *Borrelia* bacteria.

We have previously shown that the *Borrelia* Dextramer assay could identify a *Borrelia*-specific T cell response in neuroborreliosis patients compared to seronegative healthy individuals. Here, we have investigated if the assay could identify a response in healthy seropositive forest workers from Poland. Due to frequent exposure to tick bites, forest workers are particularly at high risk of Lyme borreliosis. We have investigated 19 seropositive forest workers. No T-cell response was detected in healthy seropositive forest workers compared to neuroborreliosis subjects.

These results are a promising proof of principle for the potential of the *Borrelia* Dextramer assay as a diagnostic test for Lyme disease. The clinical potential of the assay as a supplementary diagnostic test in the routine clinical practice for Lyme disease needs to be further examined in large-scale studies.

P28 **A droplet digital PCR assay for direct detection of Borrelia bacteria DNA in cerebrospinal fluid specimens**

Trine Andreasen (A, B), Jens Kjølseth Møller (A, B), Malene Bek-Thomsen (A)

(A) Department of Clinical Microbiology, Lillebaelt Hospital, Vejle, Denmark, (B) Institute of Regional Health Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

The polymerase chain reaction (PCR) technique has, due to its high sensitivity and specificity, revolutionized the diagnostics of diseases with microbiological aetiology. However, studies using PCR in the diagnostics of Lyme neuroborreliosis (LNB) have shown variable results with sensitivities ranging between 12-50%. Hence, PCR is not recommended as a routine test for diagnosis of LNB.

Novel PCR-platforms enabling digital PCR may be the future tools in diagnostics of LNB. The workflow of droplet digital PCR (ddPCR) involves generation of a water-in-oil emulsion in which aqueous droplets comprise a uniform distribution of the PCR-mix. Following PCR, the amplified fluorescence signal in each droplet is detected and the signal intensity is used to determine the fraction of PCR-positive droplets in the sample. The compartmentalization of target molecules in the ddPCR technique makes this method less prone to inhibition and more precise compared to the real-time PCR methodology.

The aim of this study is to establish and validate a ddPCR assay for specific detection of the *Borrelia burgdorferi* sensu lato complex. Furthermore, the assay will be validated for direct detection of *Borrelia* DNA in cerebrospinal fluids, without initial nucleic acid purification.

The QX200 Droplet Digital PCR system (Bio-Rad) will be used. Synthetic DNA, equivalent to part of the *Borrelia* 16S rRNA gene, and *Borrelia* reference strains cultured in MKP medium will be used for optimization of the assay protocol. A pool of cerebrospinal fluids negative of microorganisms is used as no template control (NTC) and for spiking experiments. Three different DNA isolation protocols will be compared for compatibility with the ddPCR system. The optimal ddPCR protocol will then be applied on an existing set of patient samples and the PCR result will be compared with CXCL13 and intrathecal antibody production results on the same patient. The preliminary results will be presented.

P29 **New Application of Dried Blood Spot to the Diagnosis of Relapsing Fevers**

Emilie Talagrand-Reboul (A), Anne-Charlotte Livolsi (A), Pierre Boyer (A), Antoine Grillon (A), Cathy Barthel (A), Lisa Baldinger (B), Marine Engel (B), Laurence Zilliox (B), Benoit Jaulhac (A, B), Nathalie Boulanger (A, B)

(A) EA 7290-Early Bacterial Virulence: Lyme borreliosis Group, Université de Strasbourg, CHRU Strasbourg, Fédération de Médecine Translationnelle de Strasbourg, Strasbourg, France ; (B) French National Reference Center for Borrelia, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

**Background** Relapsing fevers (RF) are caused by spirochetes belonging to the genus *Borrelia*, transmitted to human by ticks (generally *Ornithodoros* spp.) or body lice. They occur in tropical countries, mainly in Africa, but also in temperate areas like USA. Main symptoms are relapsing fevers and it may be misdiagnosed with malaria or other infectious diseases. To date, biological diagnosis of RF relies mainly on Giemsa-stained blood smears or molecular techniques on whole blood which entails a complex processing.

**Aim of the study** To facilitate the diagnosis, especially in remote areas, we tested the dried blood spot (DBS) methodology applied to the molecular diagnosis of RF.

**Material & Methods** Mice were infected by the strain *B. duttonii* complex CR2a. The follow-up of the spirochetemia was performed by microscopic examination of Giemsa-stained blood smears. In parallel, capillary blood spots were directly made on standard Whatman MM3 paper. DNA extraction from DBS was first optimized, then DNA amplification was performed using a pan-relapsing fever *Borrelia* real-time PCR. Finally, the optimized protocol was applied to human blood kept at -80°C, formerly tested positive for *B. crocidurae*, a RF *Borrelia* present in west Africa, by the same PCR method.

**Results and discussion** RF *Borrelia* DNA was successfully amplified from the DBS extracts during the course of mice bacteremia (2.10E5 to 4.10E6 spirochetes/mL).

For human blood samples, a total agreement between the DBS extraction and the whole blood direct extraction methods was obtained with a higher sensitivity than blood smear examination. *Borrelia* DNA contained in the DBS remained stable and retained its integrity after storage from 1 to 14 days at +25°C.

Given the simplicity of the preanalytical phase well-adapted to tropical medicine, this new application of DBS leading to an accurate diagnosis of RF by sensitive PCR method, may facilitate RF diagnosis.

P30 **Unconventional diagnostic tests for Lyme borreliosis: a systematic review**

Alice Raffetin, Aurélie Saunier, Kevin Bouiller, Pauline Caraux-Paz, Carole Eldin, Sébastien Gallien, Romain Jouenne, Anna Belkacem, Jérôme Salomon, Olivier Patey, Emilie Talagrand-Reboul, Benoît Jaulhac, Antoine Grillon

Centre National de Référence des Borrelia, CHRU Strasbourg, Strasbourg, France, EA 7290 Virulence Bactérienne Précoce, Université de Strasbourg, Centre Hospitalier Régional Universitaire de Strasbourg, Fédération de Médecine Translationnelle, Groupe Borréliose de Strasbourg, Strasbourg, France

**Background** Lyme borreliosis (LB) diagnosis currentlyrelies mainly on serological tests and sometimes polymerase chain reaction or culture. However, other biological assays are being developed to try to improve *Borrelia*-infection diagnosis and/or monitoring.

*Objectives:* To analyze available data on these unconventional LB-diagnostic assays through a systematic literature review.

**Methods** We searched PubMed and Cochrane Library databases according to the PRISMA-DTA method and the Cochrane Handbook for Systematic Reviews of Interventions.

We analyzed controlled and uncontrolled studies (published 1983–2018) on biological tests for adults to diagnose LB according to the European Study Group for Lyme Borreliosis or the Infectious Diseases Society of America definitions, or strongly suspected LB.

The quality of each included study was assessed with the QUADAS-2 evaluation scale.

**Results** Forty studies were included: 2 meta-analyses, 25 prospective, controlled studies, 5 prospective, uncontrolled studies, 6 retrospective, controlled studies, and 2 case reports. These biological tests assessed can be classified as: (i) proven to be effective at diagnosing LB and already in use (CXCL-13 for neuroborreliosis), but not enough yet standardized; (ii) not yet used routinely, requiring further clinical evaluation (CCL-19, OspA and interferon-α); (iii) uncertain LB-diagnostic efficacy because of controversial results and/or poor methodological quality of studies evaluating them (lymphocyte transformation test, interferon-γ, ELISPOT); (iv) unacceptably low sensitivity and/or specificity (CD57+ NK cells and rapid diagnostic tests); and (v) possible only for research purposes (microscopy and xenodiagnoses).

**Discussion** QUADAS-2 quality assessment demonstrated high risk of bias in 25/40 studies and uncertainty regarding applicability for 32/40, showing that in addition to polymerase chain reaction and serology, several other LB-diagnostic assays have been developed but their sensitivities and specificities are heterogeneous and/or under-evaluated or unassessed. More studies are warranted to evaluate their performance parameters. The development of active infection biomarkers would greatly advance LB diagnosis and monitoring.

P31 **Inflammatory Responses in Borrelia afzelii Culture Positive Patients with Early Disseminated or Early Localized Lyme Borreliosis**

Tjaša Cerar Kišek 1, Rok Blagus 2 Maša Velušček 3, Eva Ružić Sabljić 1, Franc Strle 3, Daša Stupica 3

1 Institute of Microbiology and Immunology, Faculty of Medicine Ljubljana, Ljubljana, Slovenia, 2 Institute for Biostatistics and Medical Informatics, Faculty of Medicine Ljubljana, Ljubljana, Slovenia, 3 Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia

**Background** Different *Borreliae* species differ in their potential to disseminate hematogenously in patients with early Lyme borreliosis (LB), but it is not known whether hematogenous dissemination of *Borreliae* depends also on the differences in host immune responses.

**Methods** The levels of 15 cytokines and chemokines, representative of innate, Th1, and Th17 immune responses, were assessed using bead-based Luminex multiplex assay in acute sera from 58 adult patients with multiple erythema migrans (MEM) and 76 age and sex matched patients with solitary EM at a single-centre university hospital.

**Results** At enrolment, only the levels of CXCL10 and CCL19, representatives of THh1 inflammatory response, differed significantly between patients with MEM and those with solitary EM, however the differences were just the opposite for the two mediators and did not reach statistical significance when adjusted for multiple comparisons (mean 385.88 ± 337.31 pg/mL vs 332.09 ± 392.28 pg/mL; *P*=.03, *P* adjusted=.24 and 72.18 ± 64.01 pg/mL vs 78.68 ± 46.04 pg/mL; *P*=.02, *P* adjusted=.23, respectively). Patients with MEM and those with solitary EM were pooled together for the investigation of the association between inflammatory response and the severity of acute disease, as assessed by the presence or absence of LB-associated constitutional symptoms. The levels of inflammatory mediators in 42 (31.3%) patients with LB-associated constitutional symptoms at enrolment were similar to those in 92 asymptomatic patients.

**Conclusions** Marginal differences in inflammatory responses between patients with MEM and those with solitary EM suggest that other pathogenic mechanisms might be of higher significance for controlling dissemination of early infection with *B. afzelli*, such as variations in the inherent hematogenous potential of different *B. afzelii* strains or yet unidentified host genetic predispositions. Inflammatory mediators may not be solely accountable for development of LB-associated constitutional symptoms in a subset of patients with EM who develop these symptoms.

P32 **Neutrophil extracellular traps (NETs) in the cerebrospinal fluid of children and adults with Lyme neuroborreliosis**

Appelgren D, Hedin-Skogman B, Nordberg M, Perander L, Nyman D, Nyberg C, Sjöwall C, Sjöwall J

Clinic of Infectious Diseases, Linköping University Hospital and Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Sweden.

**Background** Although neutrophils operate as part of the early innate defense in the skin and potentially may eliminate the Borrelia spirochete via phagocytosis, oxidative burst and hydrolytic enzymes, their importance in Lyme neuroborreliosis (LNB) is still obscure. Formation of neutrophil extracellular traps (NETs) is associated with the production of reactive oxygen species and consists in the extrusion of the neutrophil's own DNA, forming traps that can retain and kill bacteria. Whereas NET formation was recently studied in pneumococcal meningitis, the role of NETs in LNB has so far not been investigated. Despite being a bacterial infection, lymphocytes are the dominating cell type in the cerebrospinal fluid (CSF) of LNB patients at diagnosis. However, signs of neutrophil activation at an early stage may be overlooked. Since children with LNB often attend medical care earlier than adults do, we hypothesized that NETs as a product of neutrophil activation could be detectable in the CSF in these patients.

**Aim** To detect NETs in the CSF of children and adults with LNB.

**Patients and methods** CSF from 111 well-characterized children and 63 adults with LNB and other infections in the central nervous system (CNS) were analyzed for the presence of NETs with capture ELISA, detecting complexes of DNA and myeloperoxidase.

*Preliminary results* NETs were detectable in CSF of 14 (13%) children; eight (7%) with LNB, three (3%) with enteroviral meningitis, two (2%) with tick-borne encephalitis and one (1%) with idiopathic facial nerve palsy. One (6%) adult patient with LNB had signs of NET-formation in the CSF.

**Conclusion** We report, for the first time, evidence of NET-formation in the CSF of both children and adults with LNB as well as in children with other CNS-disorders with mononuclear pleocytosis. Further studies are needed to elucidate the role of NETs in LNB.

P33 **Cytokine responses in immunocompetent versus immunosuppressed neoehrlichiosis patients**

Linda Wass (A, B), Christine Lingblom (A, B), Lisa Labbé Sandelin (C), Peter Wilhelmsson (D), Per-Eric Lindgren (D), Pia Forsberg (D), Hanne Quarsten (E), Christine Wennerås (A, B)

A Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden, B Region Västra Götaland, Sahlgrenska University Hospital, Department of Clinical Microbiology, Gothenburg, Sweden, C Uppsala University, Uppsala, Sweden, D Department of Clinical and Experimental Medicine, Medical Microbiology, Linköping University, Linköping, Sweden, E Department of Medical Microbiology, Sørlandet Hospital Health Enterprise, Kristiansand, Norway

Introduction *Candidatus* (*Ca.*) Neoehrlichia (N.) mikurensis is a tick-borne bacterium that can give rise to a severe infectious disease named neoehrlichiosis in immunocompromised patients. However, immunocompetent persons can also become infected by this novel microbial agent. The aim of this study was to investigate if the cytokine responses engendered by *Ca.* N. mikurensis differs between immunosuppressed and immunocompetent individuals.

Material and methods Serum samples from 31 untreated neoehrlichiosis patients were analyzed for the levels of 27 cytokines by using a multiplex-based Luminex immunoassay. Nine of the patients were immunocompetent, the remaining 22 were immunosuppressed. The data were analyzed by using GraphPad to reveal differences in the patterns of cytokines.

Results The nine immunocompetent individuals who were infected by *Ca.* N. mikurensis had higher levels of Interleukin (IL)-1ra and IL-15 than the immunosuppressed patients. Conversely, the immunosuppressed patients had higher levels of the growth factor for production of neutrophils and macrophages, Granulocyte and Macrophage-Colony Stimulating Factor (GM-CSF). The immunosuppressed patients also had higher levels of monocyte chemoattractant protein-1 (MCP-1) than the immunocompetent ones, and raised levels of IL-5, IL-6, IL-10, IL-12, IL-17, IP-10 as well as fibroblast growth factor (FGF Basic).

Conclusion Differences in levels of cytokines in serum were observed between the immunosuppressed and immunocompetent patients infected with *Ca.* N. mikurensis, indicating partly dissimilar infectious defense mechanisms in these two groups of patients. Further research is needed to understand the complexity of defense against *Ca.* N. mikurensis in immunosuppressed and immunocompetent patients.

P34 **Toll-like receptors: deciphering their role toward Borrelia spp.**

Verena Mündler[1], Claire Battin[2], Michael Reiter[1], Gerold Stanek[1], Peter Steinberger[2], Hannes Stockinger[1]

[1]Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna[2,] Division of Immune Receptors and T Cell Activation, Institute of Immunology, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna

Lyme borreliosis (LB) is the most common tick-borne disease in the Northern hemisphere. In Europe the species *B. afzelii*, *B. bavariensis*, *B. burgdorferi*, *B. garinii* and *B. spielmanii* are known to cause LB. In the United States *B. burgdorferi* is – to the largest part - the sole agent of disease. Little is known about the complex interactions of innate immune cells as the first lines of defense toward these spirochaetes. Especially the sensing via pattern recognition receptors (PRRs) e.g. Toll-like receptors (TLRs) is of major interest. Hence, this project aims to shed light on the role of surface-located TLRs during the development of an early inflammatory response toward *Borrelia* spp.

Initially, the T-lymphocytic Jurkat cell line was retrovirally transduced with an enhanced green fluorescent protein (GFP) reporter gene under a NFκB-inducible promotor as well as genes for the respective TLRs. Subsequently, chosen *Borrelia* spp. strains were co-cultivated with reporter cells at different Multiplicities of Infection. After 24 hours GFP-expression was measured by flow cytometry.

Our results confirm the predominant role of TLR1/2 as a sensor toward triacylated lipoproteins. Furthermore, we were able to proof for the first time the action of TLR2/6 in detecting diacylated lipoproteins. Moreover, it was frequently speculated that TLR5 contributes in recognizing *Borrelia*-specific flagellin, which we were able to disproof. Moreover, a recently described LPS-like protein found in *Borrelia* spp. could not trigger a strong immune reaction via TLR4/CD14.

Overall, the reporter cells provide a fast and reliable screening method for various PRRs and we were able to identify relevant TLRs for the detection of *Borrelia* spp. Moreover, we were able to pinpoint major differences between the chosen *Borrelia* spp.

Supported by the PhD program Cell Communication in Health and Disease (CCHD) by the Austrian Science Fund (FWF)

P35 **Determination of the complement-inhibitory activity of different outer surface proteins of Borrelia recurrentis**

Florian Röttgerding, Eva Gerlach, and Peter Kraiczy

Institute of Medical Microbiology and Infection Control, University Hospital of Frankfurt, Paul-Ehrlich-Str. 40, D-60596 Frankfurt, Germany

*Borrelia recurrentis* is the only causative agent of Louse-borne relapsing fever (LBRF) and claimed as a „neglected arthropod-borne pathogen". One strategy of spirochetes to evade innate immunity involves inactivation of complement by interaction with complement regulators or complement components. Two complement-interacting proteins, CihC and HcpA have previously been characterized in *B. recurrentis*, but there is a cluster of additional *hcpA* homologous genes adjacent to these two borrelial genes. Thus, the goal of the present study is aimed at identifying novel immune evasion determinants that act as potential pathogenicity factors of *B. recurrentis*. Different approaches have been chosen to examine the inhibitory potential of five outer surface proteins on the alternative (AP), classical (CP) or lectin pathway (LP) of complement. In addition, hemolytic assays were employed to further assess the inactivation capacity of these borrelial proteins on the terminal pathway (TP) and CP. We further elucidate the binding properties of each protein to Factor H, Factor I, C3b, C4, and C5 by employing ELISA. Finally, the inhibitory capacity of these molecules on the TP was investigated by examining the polymerization of the late complement component C9. In summary, we were able to functionally characterize five out of nine borrelial proteins, all of which specifically inhibited the AP while one protein also terminates activation of the CP and LP. All five proteins examined bound C3b but did not interact with Factor H, Factor I or C5. Furthermore, binding to C4 could be demonstrated for the CP/LP-inactivating protein. Interestingly, four out of five proteins inhibited the TP by blocking C9 polymerization and assembly of the terminal complement complex. Taken together, our data indicate that the proteins examined display inhibitory capacity on the AP probably due to the interaction with C3b while direct binding to C4 may result in terminating CP and LP activation.

P36 **Epidemiological, clinical and laboratory characteristics of Slovenian patients with proven human granulocytic anaplasmosis**

Stanka Lotrič-Furlan 1, Miroslav Petrovec 2, Katja Strašek Smrdel 2, Tatjana Avšič-Županc 2, Franc Strle 1, Tereza Rojko 1

1 Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia, 2 Institute for Microbiology and Immunology, Medical Faculty, Ljubljana, Slovenia

**Objective** To present epidemiological, clinical and laboratory characteristics of adult patients with proven human granulocytic anaplasmosis (HGA), diagnosed at the UMC Ljubljana, Slovenia, from 1996 to 2018.

**Methods** Patients >15 years old, included in the study on the etiology of acute febrile illness occurring after a tick bite, with proven *Anaplasma phagocytophilum* infection established by positive PCR result with subsequent sequencing of the amplicons to demonstrate specific anaplasmal DNA, and/or seroconversion or a ³4-fold increase in antibody titres to *A. phagocytophilum*, qualified for the present report.

**Results** Sixty-nine patients fulfilled criteria for proven HGA. They were 55 (24 – 77) years old; 43 (62.3%) of them were males. All the patients had acute febrile illness (duration at presentation 1 – 14 days) with headache (85.5%), chills (73.9%), myalgia (60.9%) and/or arthralgia (39.1%). The most common laboratory abnormalities were elevated concentration of C-reactive protein in 67 (97.1%), thrombocytopenia in 62 (89.9%), abnormal liver function test results in 53 (76.8%) and leukopenia in 46 (66.7%) patients. Forty-five (65.2%) patients required hospitalization, 2 (2.9%) of them were treated in the intensive care unit. Fifty (72.5%) patients were treated with doxycycline. No death occurred during acute illness. The diagnosis of HGA was confirmed by positive PCR and seroconversion in 32 (46.4%) patients, by positive PCR and ³4-fold change in antibody titres in 10 (14.5%) patients; only by positive PCR in 8 (11.6%) patients, only by seroconversion in 14 (20.3%) patients and only by ³4-fold change in antibody titres in 5 (7.2%) patients. The large majority of patients were followed for at least 1 year. In all of them the outcome was favourable.

**Conclusions** In our patients with proven HGA the disease course was mostly mild to moderately severe, complications were uncommon, and the outcome was excellent.

P37 **Serodiagnosis of Lyme borreliosis – is IgM in serum more harmful than helpful?**

Henrik Hillerdal 1, Anna J Henningsson 2, 3, 4

1 Department of Paediatrics, Region Jönköping County, Sweden, 2 Department of Clinical Microbiology, Laboratory Medicine, RegionJönköping County, Sweden, 3 Department of Clinicial and Experimental Medicine, Linköping University, Sweden, 4 Department of Clinical Microbiology, Region Östergötland, Sweden

Interpretation of serological findings in suspected Lyme borreliosis (LB) may be challenging in endemic areas and IgM antibodies in serum are often associated with false positive reactivities. There is a risk for over-diagnosis of LB, inadequate use of antibiotics and potential delay of proper diagnosis. The clinical value of IgM analysis in serum in LB diagnosis is therefore questioned. The aims of this study were to investigate how well the clinical recommendations for the diagnosis of LB and when to test for *Borrelia*-specific antibodies were followed in Jönköping County, Sweden, and to evaluate the clinical value of IgM antibodies in serum. In total, 4428 *Borrelia*-specific antibody tests in serum were analyzed in Jönköping County during 2017. Of these, 643 individual patients had positive results (IgM and/or IgG), of which we had to exclude 33 patients due to inaccessible medical records. The remaining patients (n=610) with positive test results were then divided into separate groups of either IgM and/or IgG-positivity. Based on current European recommendations, we defined the criteria for correct indication for serological testing and how to evaluate the diagnosis made by the clinician. Medical records and laboratory test results for each patient were then assessed according to these criteria.

Only 183/610 (30%) of patients were tested according to the European recommendations. The groups positive for either isolated IgG or both IgM and IgG antibodies showed a similar pattern with high number of diagnoses assessed as being confident or doubtful. Isolated detection of IgM (without concomitant IgG) was only helpful in 50% of the diagnoses assessed as being confident or doubtful. Thus, 50% of the LB diagnoses in patients with isolated IgM reactivity in serum were assessed as incorrect (LB unlikely). Isolated IgM positivity in serum shows limited clinical value and needs further assessment before being reported by the laboratory.

P38 **Alpha-gal allergy after a tick bite: A case report from Austria**

Veronika Pisazka, Georg Duscher, Adnan Hodžić, Norbert Reider, Franz Allerberger

Austrian Agency for Health and Food Safety (AGES), Vienna, Austria, Institute of Parasitology, University of Veterinary Medicine, Vienna, Austria, University Hospital for Dermatology, Venereology and Allergology, Innsbruck, Austria

Tick bites can cause the alpha-gal syndrome, a delayed red meat allergy directed against mammalian galactose-alpha-1,3-galactose carbohydrate (alpha-gal). The alpha-gal epitope is expressed on cells of non-primate mammals whereas humans, apes and Old World monkeys lack the synthetic machinery to generate the alpha-gal, which can lead to a strong immune response against the glycan molecule. Bites by *Ixodes ricinus* are considered the primary cause of IgE antibody responses specific for alpha-gal in Europe. We report on a 51-year old Austrian male with recurrent delayed anaphylactic reactions after red meat consumption. Laboratory testing for IgE titers against galactose-alpha-1,3-galactose was done using ImmunoCAPTM (Thermo Fisher Scientific). A 51-year-old sporting male, with a known history of cat allergy, acquired a tick bite in Austria in spring 2017, which - within 48 hours - resulted in inflammation of the skin-area around the bite. Three months later, the patient experienced an allergic reaction approximately 8 hours after having medium rare steak for dinner. His symptoms included an itchy rash on both sides of the torso and on both arms and persisted for several hours. In spring 2018, the patient acquired another tick bite. The patient's skin reaction was similar to that of the previous year. In the following months, the patient experienced five episodes of severe allergic reactions, each during night after having beef for dinner. The symptoms included pruritic urticarial rash involving his entire body along with swollen hands, diarrhea, vomiting and in some episodes even shortness of breath. On request of the patient, an alpha-gal test was performed, revealing a significant elevation of specific IgE antibodies (>100.00 kU/L). This case report aims to raise awareness that recurrent delayed anaphylactic reactions after tick bites can occur in Austria.

P39 **Finger-Stick Whole Blood Tier 1 Lyme Assay: Method Comparison in a CLIA Setting**

I. Blandy, R. Duncan, R. Reed, S. Fall, L. Hedges, C. Charles, C. Alberto, S. Pinedo, A. King, J. Sun, R. Egan, J. McClure

Quidel, San Diego, United States

Quidel has developed the Sofia 2 Lyme FIA – a novel Tier 1 Lyme assay that uses a low volume finger-stick whole blood sample for use in point-of-care settings. Current Lyme assays require that venous samples be processed prior to running either serum or plasma on separate IgM or IgG assays, which may delay patient prognosis and treatment. The Sofia 2 Lyme FIA uses a proprietary blood collection device, in which the operator draws a low volume (25 µL) finger-stick blood sample from the patient and immediately dispenses a diluted plasma sample onto the Sofia 2 Lyme FIA test cassette. Within 15 minutes, the assay qualitatively reports the presence of IgM and/or IgG *Borrelia burgdorferi* antibodies.

Matched specimens (finger-stick and serum/plasma) were collected from subjects suspected of and exhibiting symptoms of Lyme disease in endemic regions of the U.S. The finger-stick whole blood samples were collected and processed on the Sofia 2 Lyme FIA by untrained CLIA operators. The whole blood results were compared to serum/plasma results from predicate Tier 1 and Tier 2 IgM and IgG assays.

The Sofia 2 Lyme FIA IgM has a 1st tier PPA of 82.4% and 1st tier NPA of 79.8% compared to the predicate assay. The Sofia 2 Lyme FIA IgG has a 1st tier PPA of 88.9% and a 1st tier NPA of 85.9% compared to the predicate assay. Sofia 2 Lyme FIA demonstrates excellent performance when compared to serum/plasma results of the predicate Tier 1 and Tier 2 methods. Sofia 2 Lyme FIA uses a low volume finger-stick whole blood sample that is processed in less than 1 minute, eliminating the need to process a serum/plasma sample. Sofia 2 Lyme FIA can be used in CLIA-waived settings, qualitatively reporting the presence of *Borrelia burgdorferi* IgM and IgG antibodies within 15 minutes.

P40 **Population Structure of Borrelia turcica from Greece and Turkey**

Hepner, Sabrina a, Fingerle, Volker a, Duscher, Georg Gerhard b, Felsberger, Gerit c, Marosevic, Durdica a, Okeyo, Mercy a, Sing, Andreas a, Margos, Gabriele a

a German National Reference Centre for Borrelia, Bavarian Health and Food Safety Authority, Veterinärstr. 2, Oberschleissheim, 85764, Germany, b Institute of Parasitology, Department of Pathobiology, Veterinary University of Vienna, Veterinärplatz 1, Vienna, 1210, Austria, c AGES - Austrian Agency for Health and Food Safety, Währingerstr. 25a, Vienna, 1090, Austria

*Borrelia turcica*, a member of the reptile-associated *Borrelia* clade, is vectored by *Hyalomma aegyptium*. The only suggested reservoir hosts are tortoises of the genus *Testudo*. *Borrelia turcica* has been described to occur in several Southeastern European countries including Turkey, Romania, Bulgaria and Greece but so far nothing is known about the relationship of these populations and whether or how they are structured. Using multilocus sequence typing (MLST) on eight chromosomally located housekeeping loci (*clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB* and *uvrA*) we analyzed 43 *B. turcica* isolates from Serres, Greece (n= 15) collected in 2017 and Izmir, Turkey (n= 28) collected in 2018. To understand their relationship a maximum-likelihood phylogenetic tree and goeBURST analysis were done based on MLST sequence data and allelic profiles, respectively. Relapsing fever (RF) and Lyme disease (LD) group species were used as outgroup in the phylogeny. The data we generated confirmed that the samples of *B. turcica* investigated here were divergent from LD and RF species. Within the *B. turcica* clade, samples of different geographic origin (Greece, Turkey) clustered together in terminal branches; no obvious differences between the Greek and Turkish samples were noticeable. A goeBURST diagram using triple-locus variants revealed very few clonal complexes with the majority of samples appearing as singletons. Minor clonal complexes (consisting of two sequence types) comprised only Greek isolates, only Turkish isolates or both. 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 evolution of this species.

P41 **First detection of Amblyomma variegatum and molecular finding of Rickettsia africae in Sardinia, Italy**

Elisabetta Pintore a (Presenting author), Emanuela Olivieri b, Anna Maria Floriano b, Davide Sassera b, Nino Sanna c, Luisella Succu d, Giovanni Garippa a

-a Institute of Parasitology of the Department of Veterinary Medicine of Sassari, Università di Sassari, Italy - b Department of Biology and Biotecnology of the University of Pavia, Italy - c Private Veterinary Sassari, Italy- d Tour Guide, Sassari, Italy

Here we present the first detection of *Amblyomma variegatum*, and the molecular evidence of a pathogen, *Rickettsia africae,* in Sardinia Island. The tick, the second individual of this species reported in Italy (the first was reported in Sicily by Albanese in 1971) was collected in August 2018 from the inguinal region of an adult female sheep in a farm located in Santa Giusta, a region near by Sassari (North-West Sardinia). The tick was identified under a stereomicroscope by morphological keys as an adult male of *A. variegatum* at the Institute of Parasitology of the Department of Veterinary Medicine of Sassari. The identification was confirmed by sequencing of a fragment of 12S rDNA at the Department of Biology and Biotecnology of the University of Pavia. Phylogenetic analysis shows that the collected tick 12S rDNA clusters with sequences from African *A. variegatum* individuals and, in particular, is embedded in the previously identified West African group. We tested the tick DNA for the presence of *Ehrlichia*, *Rickettsia*, *Anaplasma*, *Theileria* and *Babesia*, using published PCR protocols. The tick was found positive to *Rickettsia* and the obtained sequence matched at 100% identity with *R. africae.* The area were the tick was detected was inspected in multiple occasions, looking for other specimens of *A. variegatum*, without any results. In the same period another male specimen of *A. variegatum* was found in Haute Corse in 2019. The authors' hypothesis is that the presence of this single male specimen of *A. variegatum* is an occasional finding, probably linked to the migrating birds that cross Sardinia and Corsica from Africa during summer. Anyway, it is important to emphasize that the tick was vector of *R. africae,* thus additional monitoring must be performed as it would be a serious problem for public health if a population stabilizes in Sardinia.

P42 **Could a bacterial symbiont be a potential serological marker for the tick bite? The case of Midichloria mitochondrii in Ixodes ricinus.**

Valentina Serra, Viktoria Krey, Emanuela Olivieri, Giulia Chiappa, Chiara Bazzocchi, Alessandra Cafiso

Department of Veterinary Medicine, University of Milan, Milan, Italy; Department of Biology and Biotechnology, University of Pavia, Pavia, Italy; 3BCA-research, BCA-clinic Betriebs GmbH & Co. KG, Augsburg, Germany; Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi" and EpiSoMi Research Center, University of Milan, Milan, Italy

*Midichloria mitochondrii* is an intracellular symbiont of *Ixodes ricinus*, the most common tick species parasitizing humans in Europe. This bacterium has a prevalence of 100% in *I. ricinus* females and is also present in the immature stages. This vertically transmitted bacterium is mainly localized in the ovary of its host, but is also present in additional organs, including salivary glands. Molecular and serological evidences suggest that the transmission of *M. mitochondrii* to the vertebrate host can occur during *I. ricinus* blood meal. Tick bites remain often unnoticed due to rarity of immediate symptoms, implying the risk of developing occult tick-borne infections and leading, in turn, to a potential risk factor for the onset of chronic diseases. Since suitable tools to determine the previous exposure to *I. ricinus* bites are needed, this work was focused on evaluating the suitability of a *M. mitochondrii* protein for the diagnosis of *I. ricinus* bite. We screened 274 sera of patients exposed to *I. ricinus* bite, and collected from several European countries, using a *M. mitochondrii* flagellar protein produced in recombinant form as antigen for an ELISA assay. Our results revealed that positivity to *M. mitochondrii* protein is higher when the tick bite is considered as certain/almost certain, and lower in case of uncertainty of the bite. The highest positivity was observed in samples from patients affected by Lyme disease (47.3%) and the lowest (2%) in negative controls. According to the obtained results and on the basis of its widespread presence in *I. ricinus* individuals, *M. mitochondrii* could be regarded as a useful source of antigens for the development of a serological test for *I. ricinus* bite. Further analyses could highlight the potential role of *M. mitochondrii* proteins as an indirect epidemiological marker of exposure to *I. ricinus* in multiple vertebrate species.

P43 **Urbanization of ticks: underestimated risk of getting a tick-borne disease in popular recreational areas in Tallinn, Estonia**

Julia Geller

National Institute for Health Development, Estonia

Green areas, such as parks, recreational and leisure zones, outdoor gym sites and health trails attract citizens and tourists and provide possibilities for various outdoor activities within the city area. The development of green infrastructures, however, may also attract ticks to inhabit peri-urban and urban territories, despite scarce local floral and faunal diversity.

Although TBE incidence rate in Estonia significantly decreased and remain stable last years at 6,5 cases per 100 000 population, it is still one of the highest in Europe and keeps endemic in the most regions through the country. The incidence for Lyme borreliosis has raised to 173,6 cases per 100 000 in 2018. Of 6789 LB and 572 TBE cases registered in 2012-2016, averagely 13% of infected individuals with known possible origin of infection, reported a tick bite within urban areas.

This pilot study, conducted in 2018, aimed to reveal the presence of ticks and evaluate the risk of getting a tick bite in the popular recreational areas, outdoor sports and leisure sites located within the city and in the nearest peri-urban surroundings and to analyze taxonomic content of tick-borne pathogens.

Of over 10 000 m2 flagged and 20 sites (17 urban and 3 suburban) studied, there were 1024 ticks collected. 1 adult male tick was morphologically identified and confirmed by partial ITS2 sequencing as *I. persulcatus*, that is the first identification of *I. persulcatus* about 120 km westward of its known distribution area. In two popular family recreational areas the estimated abundance of ticks per 100 m2 were up to 25,9. Of tick-borne pathogens, the most prevalent was *Borrelia burgdorferi* s.l. (0-25%), followed by *Rickettsia helvetica* (0-33%), *Anaplasma phagocytophilum* and *Candidatus* Neoehrlichia mikurensis (0-12%) and *B. miyamotoi* (0-4%). Of four *I. ricinus* ticks, tested positive for TBEV, one was detected with TBEV-Sib.

P44 **Electrophoretic identification of allozyme variability of tick populations Ixodes persulcatus in suburban anthropogenic zones of the city of Irkutsk**

Dzhioev Yu.P.1, Yakhnenko V.M.2, Kozlova I.V.3, Doroschenko E.K.3, Lisak O.V.3, Suntsova O.V.3, Paramonov A.I.3, Savinova Yu.S.3, Kiselev D.O.1, Belyaeva A.S.1, Zlobin V.I.1

1Irkutsk State Medical University, Irkutsk, Russia, 2Limological Institute of the Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia, 3Scientific Center for Family Health and Human Reproduction Problems, Irkutsk, Russia

In suburban areas of Irkutsk, there are foci of mixed tick-borne infections: tick-borne encephalitis, borreliosis, rickettsiosis, anaplasmosis, and ehrlichiosis. The collection of ticks *I. persulcatus* took place in 2 suburban anthropogenic zones of the city of Irkutsk - in the forests along the Baikal (BT) and Goloustinsky (GT) motorways. The site for collecting ticks on BT (127 copies) is located 47 km from the city of Irkutsk and 2 km from the Irkutsk reservoir. BT is intensively used by motor transport, the area is built up by summer plots, recreation centers and hotel complexes. The site for collecting ticks on GT (13 copies) is located in the vicinity of the village of Dobrolet, located 33 km from Irkutsk. Anthropogenic load on GT is less pronounced. The direct distance between BT and GT is about 40 km. 9 enzyme systems (aconitase, diaphorase, carbonic anhydrase, creatine kinase, lactate dehydrogenase, malate dehydrogenase, superoxide dismutase, esterase, esterase D), encoded by 13 loci (AH-1, DIA-1, CA-1, CA-2 , CA-3, IC, LDH, MDH-1,2, SOD-1, SOD-2, EST-1, EST-2, ESTD). Of them in the BT sample, 8 were polymorphic, and in HT - 5. The phenotypic manifestation of the studied loci basically corresponds to the previously described homologous loci for closely related species with the corresponding genetic control. CK, LDH, EST-1, CA-2, CA-3 loci were monomorphic. The nature of the distribution of phenotypes across isolates MDH-1,2 allowed us to adopt a model of two polymorphic loci with equal frequencies of the corresponding alleles. Frequency analysis of alleles of polymorphic loci using the homogeneity test revealed significant differences between the BT and GT samples for the SOD-1 loci ($P < 0.05$) and SOD-2 ($P < 0.05$). Fixed alternative alleles were not detected. Allozyme analysis data showed a high heterogeneity of samples of ticks taken in one place and the absence of differences between samples from different geographic points.

P45 **Prevalence of pathogens in ticks collected from humans in Belgium**

Tinne Lernout, Nick De Regge, Katrien Tersago, Manoj Fonville, Vanessa Suin and Hein Sprong

Sciensano, Wageningen University, National Institute for Public Health and Environment (RIVM)

Since tick-borne diseases often present with mild and non-characteristic symptoms, it is difficult to assess their public health risk and burden. In order to evaluate the risk of human exposure to tick-borne pathogens in Belgium, a study on the prevalence of several pathogens was conducted on feeding ticks removed from humans in 2017. A citizen based collection method, using an existing notification tool for tick bites, allowed to collect a sample of ticks across the country, at low cost. Citizens were invited to send ticks detached from the skin by postal mail and to fill in an online questionnaire, to collect data on the geographical location and circumstances of the bite.

In total 1,599 ticks were microscopically differentiated for species and developmental stage and screened by PCR for the presence of the following pathogens: *Anaplasma phagocytophilum, Babesia* spp., *Borrelia burgdorferi* sensu lato (s.l.), *Borrelia miyamotoi*, *Neoehrlichia mikurensis*, *Rickettsia helvetica* and tick-borne encephalitis virus (TBEV).

The great majority of (nymphs and adult) ticks belonged to the *Ixodes ricinus* species (99%). Other tick species were *Ixodes hexagonus* (0.7%) and *Dermacentor reticulatus* (0.3%).

About 14% of the ticks in the study were infected with *Borrelia burgdorferi* s.l.. The most common species were *B. afzelii* (52%) and *B. garinii* (21%).

Except for TBEV, the other tick-borne pathogens studied were all detected in the tick sample, although at a lower prevalence, ranging between 1.5% (*Babesia* spp.) and 6.8% (*R. helvetica*). *Rickettsia raoultii*, the causative agent of tick-borne lymphadenopathy, was identified for the first time in Belgium, in two out of five *D. reticulatus* ticks.

In addition to Lyme borreliosis, Belgian clinicians should thus be aware of possible other tick-borne diseases in patients presenting fever or other non-characteristic symptoms after a tick bite.

P46 **Detection and characterisation of Borrelia and other pathogens found in ticks**

Daniel Bailey, Jackie Duggan, Julian Hiscox, Nicholas Evans, Richard Vipond, Steven Pullan

Public Health England, HPRU - Emerging Zoonotic Infections, University of Liverpool

**Background** The most prevalent tick borne disease in Europe and the United Kingdom is Lyme Disease which is caused by the bacteria *Borrelia burgdorferi*. A range of other potential pathogens such as *Anaplasma* and *Rickettsia* are also present in tick populations across the UK and pose a risk to human health. This poster summarises a number of experiments that have been performed to develop and optimise assays to detect and characterise *Borrelia* and other pathogens directly from ticks.

**Method** Ticks are identified, homogenised and then digested for nucleic acid extraction. The nucleic acid extract is tested using a panel of q-PCR assays for a range of tick borne diseases including *Anaplasma*, *Bartonella*, *Borrelia*, *Coxiella*, *Francisella*, *Rickettsia* and Tick-Borne Encephalitis virus.

Positive samples are then sequenced using amplicon (targeted) or metagenomic (non-targeted) sequencing to determine genotype. As there is a high proportion of host DNA in the sample, extracts are digested with host specific restriction enzymes to degrade the background signal for metagenomic sequencing.

**Results** A range of samples have been tested including cohorts from Iceland, Jersey and the United Kingdom. 499 ticks have currently been tested of which 44 were positive for *Anaplasma*, 27 for *Bartonella*, 91 for *Borrelia* and 17 for *Rickettsia*. 35 ticks had multiple pathogens present.

Metagenomic sequencing has also identified other tick-borne organisms including *Ehrlichia canis*, *Francisella persica* and *Midichloria mitochondrii* although host degradation using MspJI had mixed success.

**Conclusion** The techniques used in this project provide useful data to understand the prevalence of important tick-borne pathogens such as *Borrelia* in the UK. Further experiments focussing on culture and sequence optimisation will provide higher quality sequence data for future projects including a comparison of immunodominant proteins between different *Borrelia* genotypes.

P47 **Detection of pathogens in UK ticks using molecular inversion probes**

Mia White, Richard Vipond, Jolyon Medlock, Steven Pullan, Ed Feil

Public Health England (Porton Down), University of Bath

Ticks are vectors of many pathogens worldwide, they feed on a wide range of hosts, including humans. In the UK, the main tick borne pathogen of public health importance is Lyme borreliosis (Lyme disease), one of the principal vectors of this is the most commonly found species of tick in the UK: *Ixodes ricinus*. Every year, there are about 17,000 samples screened for Lyme disease from patients presenting with febrile illness and a confirmed history of tick bite. However, the majority of these test negative, indicating there are other causative pathogens in UK tick populations. At present, there are several competent vectors of *Borrelia burgdorferi* sensu lato complex (causative agent of Lyme disease), as well as other pathogens such as *Rickettsia* and *Babesia.* Currently, the most common molecular methods to detect these are by PCR or ELISA, however, these only confirm the presence or absence of a target. This project aims to develop a panel of molecular inversion probes (MIPs) that specifically target informative genomic regions and will be used to detect and characterise multiple pathogens in ticks, species of tick and source of their last blood meal in a time and cost effective way, detecting target DNA in a single reaction tube using only commercially available reagents. This probe panel can be used on both individual and pooled ticks and will give an insight into what is present in UK ticks, their host preference, disease spread and reservoir hosts, as well as being a useful tool to screen field collected ticks for non-native species and imported pathogens to UK tick populations.

P48 **First case of imported B. miyamotoi infection in Austria**

Selma Tobudic, Mateusz Andrzej Markowicz, Heimo Lagler, Stefan Winkler, Gerold Stanek, Heinz Burgmann

Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Medical University of Vienna

*Borrelia miyamotoi* is being increasingly documented from the world's northern hemisphere. This spirochaete has been recorded in Canada as well on the east and west coasts of the United States (US). It has also been observed in numerous European countries (including the Czech Republic, Denmark, Estonia, France, Germany, Netherlands, Norway, Poland, Romania, Sweden and Switzerland) as well as in Russia through to Japan

This is a first report of imported *B. miyamotoi* infection in Austria.

A 51-year-old woman with medical history of rheumatoid arthritis treated with Rituximab presented to the outpatient clinic with symptoms of relapsing fever lasted from 3 months. Fever episodes occurred every five days with duration between two to three days. Four weeks before the onset of symptoms our patient travelled to the USA for 3 weeks. As tourist she visited east and west coast and stayed in several nationals parks of the USA. She reported about several insect bites and one tick-bite without erythema migrans.

Routine laboratory tests performed were normal, except for the evidence of leukopenia with 3.3. G/L and elevated C-reactive protein of 3. 5 mg/dl.

No abnormal findings were observed on physical examination; in particular, there was no apparent rash on the skin.

Blood and urine cultures were negative. We performed a broad-range bacterial PCR and Borrelia species was identified as pathogen microorganism. Spirochaetes were also detected in the peripheral blood smear. *Borelia miyamotoi* was identified with PCR assay of whole blood using primer specific for 16S–23S ITS regions, 16S ribosomal RNA and for the glpQ genes.

Patient was treated successfully with doxycyclin 200 mg per day for two weeks.

P49 **Tick-borne pathogens detected in blood of immunosuppressed Norwegian patients living in an endemic area**

Quarsten H a, Salte T b, Lorentzen ÅR c&d, Widding Hansen IJ e, Hamre R b, Forselv KJN c, Wennerås C f, and Noraas S a

a Sørlandet Hospital, Department of Medical Microbiology, Kristiansand, Norway, b Sørlandet Hospital, Department of Clinical Medicine, Kristiansand, Norway, c Sørlandet Hospital, Department of Neurology, Kristiansand, Norway, d Sørlandet Hospital, The Norwegian National Advisory Unit on Tick-borne diseases, Arendal, Norway, e Sørlandet Hospital, Department of Rheumatology, Kristiansand, Norway, f University of Gothenburg, Department of Infectious Diseases, Göteborg, Sweden

Tick-borne infections (TBI) are endemic on the southern coast of Norway. Although ticks harbour other bacteria than the well-known *B. burgdorferi* s.l., reports of TBIs other than Lyme borreliosis are few in Norway. Immunosuppressed patients are at risk of infections, including TBI. The aim of this study was to evaluate the occurrence of different types of TBI in the blood of patients with impaired immune function regardless of suspected infection or not. The patients (n=165) having medical conditions requiring immunosuppressive medication were included in the study during two periods, Sept. - Dec. 2018 (n=146) and March- May 2019 (n=19). All were attending hospital for treatment/control of their illness except for one who was referred to hospital with fever of unknown origin.

Whole blood samples were examined for the presence of DNA from *B. burgdorferi* s. l*., B. miyamotoi*, *A. phagocytophilum*, *Rickettsia* spp*.,* and *Candidatus* Neoehrlichia mikurensis by real-time PCR. Pathogen DNA was detected in 9.1% (15/165) of the patients (one *B. burgdorferi* s. l., one *Rickettsia* spp. and 13 *Ca.* Neoehrlichia mikurensis DNA). Two patients presented with acute illness. The first one had neoehrlichiosis with recurrent high fever, malaise and presumed thrombophlebitis and the second one had fever, increasing CRP and *B. burgdorferi* s. l. DNA detected in blood. All except for two asymptomatic patients (*Rickettsia* and *Ca.* Neoehrlichia mikurensis DNA+) were pathogen-positive in follow-up samples and were consequently treated with doxycycline. Among the treated patients, six self-reported no symptoms at the time of inclusion, whereas seven reported new health complaints during the preceding four months. Five patients experienced relief of health problems as fever, fatigue and facial rash after treatment with antibiotics.

The knowledge of the implications of TBIs in immunosuppressed patients is poor both regarding occurrence of symptomatic and asymptomatic infections and risk of severe complications in untreated patients.

P50 **Neuroborreliosis in north –eastern Poland - tips for the practitioners**

Joanna Zajkowska

Department of Infectious Diseases and Neuroinfections Medical University in Białystok

The north-eastern part of Poland is an endemic area of tick borne diseases including Lyme borreliosis. Since last decade of 20th century its incidence has been gradually increasing.

Various manifestations of neuroborreliosis, occurring solitarily or as a group of symptoms remain a significant challenge for clinicians. Lack of specific and sensitive test for Lyme disease implicate difficult and multistep diagnostic process. Final identification of the disease depends on divers laboratory tests and experience of the physician.

The diagnosis of neuroborreliosis is based on medical history, routine cerebrospinal fluid analysis and identification of antibodies directed against *Borrelia burgdorferi* in blood serum and cerebrospinal fluid. This diagnostic tool has been available in Poland for several years already. After introduction of intrathecal synthesis (AI) of anti-*Borrelia burgdorferi* antibodies test after 2007 the diagnosis is even more efficient.

The aim of the study was the assessment of incidence of neuroborrelisis, its symptoms and neurological disorder in patients diagnosed over the last 23 years according to novel diagnostic methods. Two groups of patient were analysed. Members of the first group were hospitalized before AI introducing, the second group after. Once the AI has been introduced the hospitalisation rate decreased and is nowadays at a constant level.

Consequently, the incidence of neurological symptoms similar to MS had decreased.
Common, rare and casuistic presentations are introduced as well as tips for the practitioners.
Common presentation can be based on typical clinical picture (like erythema migrans presence) of Bannwarth syndrome.

P51 **The LymeProspect KIDS study into long-term effects of Lyme borreliosis in children**

Anna D. Tulen 1*, Hedwig D. Vrijmoeth 1, 2*, Jeanine Ursinus 1, 3*, Margriet G. Harms 1, Lotte Haverman 4, Hans Knoop 5, Stefanie Henriet 6, Michaël Boele van Hensbroek 7, Barend van Kooten 8, Kristin Kremer 1, Hein Sprong 1, Tizza P. Zomer 8, Stefanie A. Gauw 3, Yolande M. Vermeeren 8, Leo A.B. Joosten 2, Bart-Jan Kullberg 2, Joppe W.R. Hovius 3, and Cees C. van den Wijngaard 1

* Authors contributed equally to this work

1 Center for Infectious Disease Control, National Institute for Public Health and Environment (RIVM), Bilthoven, the Netherlands, 2 Department of Internal Medicine & Radboud Center for Infectious Diseases (RCI), Radboud university medical center, Nijmegen, the Netherlands, 3 Department of Internal Medicine, Division of Infectious Diseases & Center for Experimental and Molecular Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands, 4 Psychosocial Department, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands, 5 Department of Medical Psychology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands, 6 Department of Pediatrics(...)

**Introduction** Approximately 5-20% of adult patients treated for Lyme borreliosis report disabling persistent symptoms. Although children may also experience persistent symptoms, pediatric studies on long-term effects of Lyme borreliosis are scarce. The aim of the ongoing LymeProspect KIDS study is therefore to prospectively assess the Health Related Quality of Life (HRQOL) and the prevalence of persistent fatigue symptoms and in children. Moreover, potential determinants for development of these persistent symptoms are investigated.

**Methods** We include children (0-17 years of age) with physician confirmed erythema migrans (EM) or disseminated Lyme borreliosis manifestations at the initiation of their antibiotic treatment. The HRQOL and the presence of persistent severe fatigue (≥6 months) is assessed by validated online questionnaires (PedsQL, PedsQL–fatigue, CIS-fatigue) every 3 months during one year follow-up. In a subset of patients, blood is collected at inclusion and after 6 weeks. Potential determinants of persistent fatigue symptoms and lower HRQOL are assessed by serology, cellular immune responses, polymerase chain reaction (PCR) for *Borrelia* and other tick-borne pathogens, and questionnaires on symptoms, disabilities, comorbidity and cognitive-behavioral variables (SDQ, IPQ).

**Preliminary results** Between July 2017 and April 2019, 39 children were included, 19 girls and 20 boys with a median age of 7 years (range 1-17). Most children (28/39; 72%) were diagnosed with EM, other children were diagnosed with Lyme neuroborreliosis (6/39; 15%), Lyme arthritis (4/39; 10%) and borrelial lymphocytoma (1/39; 3%). Available preliminary results of the study will be presented, including clinical data and laboratory outcomes.

**Conclusions** The LymeProspect KIDS study will provide insights into the long-term effects of Lyme borreliosis and determinants for persistent fatigue symptoms and lower HRQOL in children. If prevalence and risk factors for the development of persistent symptoms are different for children than for adults, specific diagnostic and treatment strategies for children are needed.

P52 **Lyme borreliosis - a scientific approach to reduce diagnostic and therapeutic uncertainties (BorrSci). An update.**

Harald Reiso, on behalf of the project group

Norwegian National Advisory Unit on Tick-borne Diseases, Haukeland University Hospital, Oslo University Hospital, Norwegian Institute of Public Health, Sørlandet Hospital Trust, Møre & Romsdal Hospital Trust

The main purpose of the project is to reduce uncertainties in diagnosing and treating disseminated Lyme borreliosis.

The Project has five work packages (WPs):

1. Increase knowledge about the phenomenon chronic Lyme disease (more below)

2. Improve treatment of neuroborreliosis (more below)

3. Establish a Norwegian Biobank for Tick-borne diseases

4. Search new biomarkers of borreliosis, and gain insight into pathogenesis and long-term complaints in neuroborreliosis

Task 1. - Seek new biomarkers for neuroborreliosis

Task 2. & 3. - Increase knowledge about prognosis of neuroborreliosis looking at:

- Genetics and immune responses (more below)

- CNS imaging by MRI & functional assessments/neuropsychology

5. Improve treatment paths, distribute new knowledge, and increase co-operation & networking

The project started September 1st 2015. The status of September 2019 will be presented, with scientific production this far, and sketches for future publications.

More about tasks in WP 1:

1. Epidemiology: prevalence of pCLD in Norway

2. Clinical: clinical characteristics of pCLD

3. Laboratory: laboratory signs of previous or ongoing infection with *Borrelia burgdorferi* or other tick-borne microbes, in pCLD

The research question in WP 2: Is two weeks of per oral doxycycline treatment (currently suggested treatment) at least as effective as six weeks doxycycline treatment in neuroborreliosis?

More about WP 4 tasks 1 & 2:

- By looking at the longitudinal profile of antibody responses we hope to discern patterns of immune reactivity of diagnostic and prognostic value.

- The patterns of reactivity in cytokines and chemokines associated with innate and adaptive immune responses will be studied.

- Since immunological and inflammatory processes depend on the patients' immunogenetic profiles, we will look at the evolving reactivity in immune response genes.

May other Research Groups in Europe contribute to genome-wide association studies (GWAS) in neuroborreliosis? Preferably 1000 persons with confirmed diagnoses of neuroborreliosis should be included in such analyses, BorrSci has 120.

P53 **Non-specific symptoms in children referred to a Lyme center**

T.P. Zomer 1, R.A. Bruinsma 1, 2, T. van Bemmel 1, 3, G.W. Landman 1, 3, B.C. van Hees 1, 4, B. van Kooten 1, 5, Y.M. Vermeeren 1, 3

1 Lyme Center Apeldoorn, Gelre Hospital, Apeldoorn, the Netherlands, 2 Department of Pediatrics, Gelre Hospital, Apeldoorn, the Netherlands, 3 Department of Internal Medicine, Gelre Hospital, Apeldoorn, the Netherlands, 4 Department of Medical Microbiology and Infection Prevention, Gelre Hospital, Apeldoorn, the Netherlands 5 Department of Neurology, Gelre Hospital, Apeldoorn, the Netherlands

Non-specific persistent symptoms in children suspected of Lyme borreliosis (LB) are challenging for clinicians. Online questionnaires circulate on non-specific symptoms and LB. This suggests, non-specific symptoms are indicative for LB. We assessed whether non-specific symptoms are more prevalent among children with positive IgG serology or a history of LB.

We included children (<18 years) suspected of LB who visited our Lyme center between 2008-2017. Serum samples were taken and questionnaires on non-specific symptoms completed. Symptoms that occurred sometimes/often/always in >50% of children were analysed. Symptoms were considered present when the frequency was often/always and the severity strong/extreme. A history of LB was defined as previous (multiple) erythema migrans, borrelial lymphocytoma, Lyme arthritis, or Lyme neuroborreliosis for which the child was treated. The prevalence of non-specific symptoms was compared using the chi-square and Fisher's exact tests.

Included were 149 children (66% female, median age 13 years); 29 (19%) had positive IgG serology; 36 (24%) had a history of clinical LB; 12 (8%) had both. Seventeen symptoms were analysed. The most common were sleep disturbances (58%), severe fatigue (57%), and headache (42%). The prevalence of non-specific symptoms was similar in children with positive versus negative IgG serology. None of the non-specific symptoms occurred more frequently in children with previous LB compared to children without. Less prevalent in children with versus without previous LB were sleep disturbances (40 vs. 66%, P<0.01), concentration problems (25 vs. 43%, P<0.05), dizziness (15 vs. 38%, P<0.01), stomach ache (17 vs. 33%, P<0.05), muscle pain (10 vs. 32%, P<0.01), tingling (6 vs. 34%, P<0.001), and unexplained sweats (25 vs. 8%, P<0.05).

Non-specific symptoms were not more prevalent in children with positive versus negative IgG serology or in children with versus without previous LB. This suggests, questionnaires on non-specific symptoms are not useful in clinical practice in Lyme centers.

P54 **Erythema Migrans: Course and Outcome in Patients Treated with Inhibitors of Tumor Necrosis Factor-alfa (TNF-α)**

Vera Maraspin 1, Petra Bogovič 1, Tereza Rojko 1, Katarina Ogrinc 1, Eva Ružić-Sabljić 2, and Franc Strle 1

1 Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia, 2 Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Slovenia

**Objectives** The aim of the study was to evaluate the course and outcome of erythema migrans (EM) in adult patients receiving TNF-α inhibitors for their underlying disease.

**Patients and Methods** Information obtained from database on adult patients diagnosed with EM from 2009–2018 revealed 16 patients with EM, 9 females and 7 males, aged 57 (33–71) years, who were receiving TNF-α inhibitors (adalimumab, infliximab, etarnecept, golimumab) for rheumatic (13 patients) or inflammatory bowel disease (3 patients). Findings in this group were compared with findings in 32 sex-, age-, and antibiotic treatment-matched immunocompetent patients, diagnosed with EM at our institution in the same year.

**Results** Fifteen (93.8%) patients presented with solitary EM, one (6.3%) with multiple EM. In comparison with control group immunocompromised patients had shorter incubation period (7 vs.14 days; p=0.0153), smaller diameter of EM (10.5 vs. 15.5 cm; p=0.0014), more often comorbidities other than immune mediated disease (62.5% vs. 25%, p=0.0269), and more frequently symptoms/signs of disseminated Lyme borreliosis (18.8% vs. 0%, p=0.0324), abnormalities at physical examination (37.5% vs. 0%, p=0.0007), and increased ESR (37.5% vs. 10.3%, p=0.0499). Treatment failure was found in 4/16 patients with impaired immunity but in none of the control group (25% vs. 0%, p=0.0094). After re-treatment with an alternative antibiotic the clinical course of Lyme borreliosis was smooth and the outcome at one year follow-up examination was favourable.

**Conclusions** Continuing TNF inhibitor during concomitant borrelial infection and using identical antibiotic treatment approaches as for immunocompetent patients resulted in more frequent EM treatment failure in patients receiving TNF inhibitors than in immunocompetent patients. However, the majority of treatment failures were mild (of 4 patients with treatment failure 3 had still visible EM >2 months after the onset of antibiotic treatment), and the course and outcome of Lyme borreliosis after re-treatment with antibiotic was favourable.

P55 **Hospitalizations for Lyme borreliosis in Belgium, 2008-2016**

Laurence Geebelen 1, 2, Philippe Beutels 3, Brecht Devleesschauwer 1, 4, Katrien Tersago 1, Niko Speybroeck 2, Tinne Lernout 1

1 Scientific Directorate of Epidemiology and public health, Sciensano, Brussels, Belgium. 2 Institute of Health and Society (IRSS), Université catholique de Louvain, Brussels, Belgium. 3 Centre for Health Economics Research & Modelling Infectious Diseases (CHERMID), Vaccine & Infectious Disease Institute, Faculty of Medicine & Health Sciences, University of Antwerp, Wilrijk, Belgium. 4 Department of Veterinary Public Health and Food Safety, Ghent University, Merelbeke, Belgium.

*Background:* A thorough analysis of hospitalizations related to Lyme borreliosis (LB) manifestations is currently lacking in Belgium. Yet, such data are essential to assess the current health and economic burden, and whether this burden is increasing. This study aims to fill that gap by using Belgium's national hospitalization database.

*Materials and Methods:* Data on hospitalizations with LB as the primary diagnosis were retrieved from the Minimal Clinical Data registry, a mandatory registry for all Belgian hospitals, using ICD-9 (2008-2014) or ICD-10 (2016) coding. Besides the number of hospitalizations, length of stay and patient demographics, available for all years, more detailed information on manifestations (through ICD-10 codes), as well as on secondary diagnoses, treatment, laboratory tests and procedures were available for the year 2016.

*Results:* In the period 2008-2016, on average 283 hospitalizations per year were recorded with LB as the primary diagnosis. The incidence ranged from 2.1/100.000 inhabitants in 2008 to 2.9/100.000 in 2010 (including re-admissions). Hospitalizations occurred proportionately more often for men (55%) than women and the highest incidence was observed in the age group of 5-14 year olds (5.1/100.000). In 2016 (n=286), of the admissions coded with a specific manifestation (n=169), 88% were coded with neurological disorders and 11% with arthritis. The mean length of stay for both manifestations was 7.2 and 4.5 days, respectively. At least one antibiotic treatment recommended for LB was administered during 66% of these hospital stays, 81% of which received intravenous antibiotics (mostly ceftriaxone). Out of the 286 admissions, 17 were re-admissions.

*Discussion:* The incidence of LB hospitalizations did not increase between 2008-2016. ICD-10 coding provides more detailed information on clinical manifestations, but coding is incomplete and not yet optimally used. To allow comparison between countries, agreements should be reached on how to use primary and secondary diagnosis codes to identify LB hospitalizations.

P56 **CXCL13 in Laboratory Diagnosis of Lyme Neuroborreliosis – evaluation of the ReaScan CXCL13 assay on fresh, frozen and cold-stored CSF samples**

Anna J Henningsson1 a,b,c, Malin Lager a, Paula Gyllemark d, Gärda Andersson a, Oskar Ekelund e, Martin Sundqvist f

a Clinical Microbiology, Region Jönköping County, Sweden; b Department of Clinical and Experimental Medicine, Linköping University, Sweden; c Clinical Microbiology, Region Östergötland, Linköping, Sweden; d Infectious Diseases, Region Jönköping County, Sweden; e Clinical Microbiology, Region Kronoberg, Växjö/Karlskrona, Sweden; f Clinical Microbiology, Örebro University Hospital, Sweden

The diagnosis of definite Lyme neuroborreliosis (LNB) requires neurological symptoms, pleocytosis in the cerebrospinal fluid (CSF) and intrathecal production of *Borrelia*-specific antibodies; *i.e.* elevated antibody index (AI). However, in early disease, *Borrelia*-specific antibodies may be absent and the sensitivity of AI may thus be limited. The B cell-attracting chemokine CXCL13 has been shown to be elevated in the CSF of patients with early LNB, and to decrease rapidly after antibiotic treatment. Thus, analysis of CXCL13 in the CSF may be helpful in AI-negative patients with possible early LNB, and as a marker for active disease. We evaluated the diagnostic performance of the rapid ReaScan CXCL13 assay (Reagena) on fresh, frozen and cold-stored CSF samples from patients with suspected LNB and compared the results with the recomBead CXCL13 assay (Mikrogen).

Test panel A: 116 CSF samples from patients investigated for LNB were retrospectively included: 32 definite LNB, 11 possible LNB with CSF pleocytosis but normal *Borrelia*-specific AI, and 73 non-LNB patients without CSF pleocytosis. CSF samples had been stored at -20oC. Test panel B: 50 fresh CSF samples from patients investigated for LNB and with CSF pleocytosis were included: 17 definite LNB, 2 possible LNB and 31non-LNB. All CSF samples were analysed for CXCL13 by ReaScan CXCL13 and recomBead CXCL13. Cut-off levels suggested by the manufacturers were applied. In addition, CSF samples were stored in refrigerator for 1, 3, 7 and 14 days respectively, before analysis with the ReaScan assay.

In panel A, the sensitivity and specificity for the ReaScan assay were 77% and 100%, and for the recomBead assay 86% and 100%. In panel B, the sensitivity and specificity for the ReaScan assay were 79% and 94%, and for the recomBead assay 84% and 84%. Cold-storage and freeze-thaw cycles affected the CXCL13 levels, especially when moderately elevated.

P57 **Retrospective comparison of two chemokine CXCL13 assays for the diagnosis of Lyme neuroborreliosis on a representative sample from a Dutch hospital**

Tamara van Gorkom a,b, Gijs H.J. van Arkel b, Willem Voet c, Steven F.T. Thijsen a, Kristin Kremer b

a Department of Medical Microbiology and Immunology, Diakonessenhuis Hospital, Utrecht, the Netherlands, bCentre for Infectious Diseases Research, Diagnostics and Laboratory Surveillance, Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands; c Department of Neurology, Diakonessenhuis Hospital, Utrecht, the Netherlands

**Introduction** Diagnosis of Lyme neuroborreliosis (LNB) relies on the detection of intrathecally produced *Borrelia*-specific antibodies. However, low antibody levels in the early phase of LNB on the one hand, and lifelong persistence of antibodies on the other hand hamper an accurate diagnosis. Thus, new diagnostic markers, with a high sensitivity in the early phase of LNB and a shorter turnaround time, are needed. Recent studies have shown that the B-cell chemokine (C-X-C motif) ligand 13 (CXCL13) can be useful in the diagnosis of early LNB.

**Methods** Two assays were evaluated for the detection of CXCL13 in cerebrospinal fluid (CSF): the Quantikine CXCL13 ELISA (R&D Systems, Minneapolis, MN, USA) and the Luminex-based *recom*Bead CXCL13 assay (Mikrogen GmbH, Neuried, Germany). Both assays were retrospectively performed on CSF of all consecutive patients for which CSF-blood sample pairs were collected between 2013-2016. Patients were divided in six groups based on the likelihood of having LNB or another disease. Receiver operating characteristic (ROC) curve analysis was performed in which the CXCL13 concentrations of definite LNB patients were compared with those of non-LNB infectious and non-infectious disease patients.

**Results** 152 consecutive patients were included, supplemented with six additional definite LNB patients; 7/158 (4.4%) were classified as definite, 11/158 (7.0%) as probable, 24/158 (15.2%) as possible LNB, 15/158 (9.5%) as non-LNB infectious, and 47/158 (29.7%) as non-infectious disease patients, and 54/158 (34.2%) as unknown disease patients. ROC curve analysis showed that the Quantikine CXCL13 ELISA and the *recom*Bead CXCL13 assay performed equally well to diagnose LNB (area under the curve: 0.985 and 0.988, respectively) (*P* value = 0.628). Both assays had a sensitivity of 100.0% and a specificity of 96.8% for detecting LNB.

**Conclusion** This study showed that CXCL13, irrespective of the assay used, is a useful tool for the diagnosis of LNB in our hospital population.

P58 **Tick-Borne Encephalitis Virus and Lyme Borrelia Causing Coinfection or Coincidence: Dilemma in Clinical Practice**

Maša Velušček, Tjaša Cerar Kišek, Eva Ružić Sabljić, Tatjana Avšič-Županc, Daša Stupica

Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia, Institute of Microbiology and Immunology, Faculty of Medicine Ljubljana, Ljubljana, Slovenia, Faculty of Medicine Ljubljana, Ljubljana, Slovenia

**Key words** Tick-borne encephalitis; Lyme borreliae; Coinfection

**Background** Information is lacking on how to manage patients with tick-borne encephalitis (TBE) virus infection and borrelial antibodies in serum indicating possible coinfection with Lyme borrelia (CLB), but missing clinical or microbiological criteria for proven CLB.

**Methods** Prevalence as well as clinical implication of proven or possible CLB as opposed to TBE alone were studied retrospectively in 690 patients with TBE, who had been hospitalized at a single medical centre from 2007 through 2013. Clinical outcome at 12 months after admission was assessed. In patients with possible CLB, clinical outcome was assessed depending on whether anti-borrelial therapy was prescribed or not.

**Results** Out of 690 patients with TBE, 386 (55.9%) patients had no clinical or microbiological indication of borrelial coinfection. Proven CLB was established in 61 (8.8%) patients: 46 patients had microbiologically proven Lyme neuroborreliosis (intrathecal synthesis of borrelial IgM or IgG antibodies in 42, borreliae isolated from cerebrospinal fluid in 5), 12 patients had concomitant erythema migrans, and 3 patients had borreliae isolated from blood. In 243 (35.2%) patients possible CLB was diagnosed. The severity of acute illness as well as the proportion of patients with incomplete clinical outcome at the 12-month follow-up were comparable among patients with TBE and proven CLB (10/27, 37.0%), TBE and possible CLB (42/129, 32.5 %), or TBE alone (43/136, 31.6 %), $P$=0.87. At the 12-month follow-up, 29/97 (30.5%) patients with possible CLB who were prescribed anti-borrelial therapy experienced incomplete clinical outcome versus 13/32 (37.5%) who did not receive antibiotics ($P$=0.47).

**Conclusions** The proportion of proven CLB in patients with TBE was considerable, but even more so the proportion of possible CLB. Neither proven nor possible CLB were associated with the severity of acute illness or long-term outcome of TBE. The benefit of antibiotic treatment in patients with TBE and possible CLB is dubious.

P59 **Complex CSF Diagnostics of Tick-borne Neuroinfections**

Ondřej Sobek 1, Lenka Hajduková 1, 2, Zuzana Bílková 1, Jan Mareš 3

1 Laboratory for CSF and Neuroimmunology, Topelex, Prague, Czech Republic, 2 Department of Neurology, Military University Hospital Prague, Czech Republic, 3 Department of Neurology, University Hospital, Olomouc, Czech Republic

Precise laboratory diagnostics of CSF in tick-borne neuroinfections is the key to a timely and successful therapy. Identification of CSF inflammations and stage determination, or more precisely, determination of the floridity of the inflammatory process in the CSF compartment, has in these cases a major clinical significance. An overview of laboratory findings in CSF in selected tick-borne neuroinfections is presented.

Basic laboratory parameters in CSF include:

- quantitative and qualitative cytology provides relevant information about presence and type of inflammatory cellularization

- biochemical and energetic parameters (Total protein, glucose, lactate and calculation of Coefficient of energy balance)

For wider differential diagnostics of serous CNS inflammations, where neuroborreliosis and TBE belong as the most common tick-borne neuroinfections in Central Europe, advanced CSF laboratory parameters need to be included:

- humoral immunological markers (cytokines - CXCL13) - detection of i.t. synthesis specific antibodies (incl. total IgG, IgM, Albumin) - Direct proof of microbial agents (PCR) In cases of specific clinical requirements further investigations can be indicated. Ranked among such laboratory investigations are oligoclonal bands using isoelectric focusing method, structural proteins of CNS for tissue damage evaluation, and possibly also autoantibodies within the frame of differential diagnostics of infectious and autoimmune processes.

It is necessary to emphasize that all laboratory investigations of CSF (e.g. cytological, biochemical, immunological and microbiological) should be understood as an integral part of complex clinical diagnostics - optimally in the frame of close cooperation between a clinician and a specialized multidisciplinary CSF laboratory. Only such a laboratory can ensure adequate technical equipment as well as a team of highly experienced and erudite personnel. Needless to say that a complex interpretation of all laboratory parameters in a concluding report should be a common standard in proper clinical diagnostics.

P60 **The United State of Borrelia**

Gabriele Margos1, Alex Gofton2, Daniel Wibberg3, Alexandra Dangel1, Durdica Marosevic1, Siew-May Loh2, Charlotte Oskam2, Volker Fingerle1

1Bavarian Health and Food Safety Authority and National Reference Center for Borrelia, Oberschleissheim, Germany, 2Vector & Waterborne Pathogens Research Group, School of Veterinary & Life Sciences, Murdoch University, South St, Murdoch, Australia, 3Cebitec, University of Bielefeld, Bielefeld, Germany

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. In 2016 an additional group of spirochetes was described from Australia being associated with Echidna and its associated ticks. In our analysis, we use genomic Information of Lyme borreliosis spirochetes, relapsing fever spirochetes, reptile associated spirochetes and the new group of Echidna associated spirochetes to re-analyse the genus boundaries of this group. 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 show that RF group species, LB group species and two species belonging to intermittent clades, *Borrelia turcica* Güner et al. 2004and *Candidatus* Borrelia tachyglossi Loh et al. 2017 all belong into a single genus. Using this method we compared taxa belonging to other genera within the order Spirochetales to confirm that POCP values above 50 % are very well suited to demarcate genus boundaries. 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.

P61 **Detection and Multi-Locus Sequence Typing (MLST) of Borrelia burgdorferi sensu lato strains in ticks collected in Vienna, Austria**

Theresa Stelzer, Anna-Margarita Schötta, Michael Reiter, Hannes Stockinger and Gerold Stanek

Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria

*Ixodes ricinus,* a known vector for *Borrelia burgdorferi* sensu lato (s.l.), is the most common tick in Austria. The *B. burgdorferi* s.l. complex currently consists of 22 genospecies including eight known causative agents for Lyme borreliosis, namely *B. afzelii, B. garinii, B. bavariensis, B. burgdorferi* sensu stricto*, B. spielmanii, B. bisettii, B. valaisiana* and *B. mayoni*. With exception of *B. bisettii* and *B. mayonii,* all of the aforementioned species have been detected in ticks collected in Austria.

By flagging we collected ticks from public parks and forests in Vienna (Austria). These ticks were screened for the presence of microbial, particularly borrelial, DNA by reverse line blot (RLB) hybridization which allows for detection of multiple tick-borne microorganisms at once. Specimens harboring only a single *Borrelia* genospecies were then genotyped by multi-locus sequence typing (MLST). Using the eBURST algorithm, obtained genotypes and genotypes available from a public database (PubMlst.org) could be put into an evolutional and geographical context.

Preliminary results from 60 ticks collected in Vienna in 2013 revealed infection rates of 8.3% for *B. burgdorferi* sensu stricto, 6.7% for *B. afzelii*, 3.3% for *B. garinii/B. bavariensis* and 3.3% for *B. valaisiana*. Three ticks showed a co-infection with multiple *Borrelia* strains and were excluded from MLST analysis.

The present, still ongoing, study will provide valuable information regarding borrelial infection rates of ticks from an urban area. Further analysis will then shed light onto population structure and epidemiology and will provide insight into the phylo-geographical and ecological evolution of the different genospecies in Vienna. In subsequent studies these data can be linked to data acquired from patient material and thereby reveal the infectious potential of certain genotypes circulating in the environment.

P62 **Multilocus sequence typing of Borrelia burgdorferi sensu lato strains detected in clinical samples**

Anna-Margarita Schötta, Theresa Stelzer, Michael Reiter, Verena Mündler, Mateusz Markowicz, Johanna Strobl, Anna Gindl, Georg Stary, Hannes Stockinger and Gerold Stanek

Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna

*Borrelia burgdorferi* sensu lato is a complex of 22 genospecies that currently harbours eight known causative agents of Lyme borreliosis, namely *B. afzelii, B. garinii, B. bavariensis, B. burgdorferi* sensu stricto*, B. spielmanii, B. bisettii, B. valaisiana* and *B. mayonii*. Infection with these agents can lead to various disease manifestations affecting skin (erythema migrans), joints (arthritis), the nervous system (Lyme neuroborreliosis) or the heart. Lyme borreliosis is common in Austria. Detection of the pathogen can be achieved by PCR from clinical samples in particular from skin biopsies and synovial fluid. In an ongoing study we are characterizing borrelial DNA from PCR positive specimens in more detail.

Presence of borrelial DNA in a given specimen was determined using two different realtime PCRs. Positive samples were then subjected to multilocus sequence typing (MLST), a technique employing the sequences of eight borrelial housekeeping genes for determination of the specific genotype. Isolates characterized by MLST can then be compared to each other and to a publicly available MLST database (PubMlst.org). Moreover, population structure analysis can be performed based on the obtained MLST profiles. Until now 33 positive samples were collected for further genetic analysis. Preliminary results revealed the presence of previously unknown alleles and sequence types in patient samples. So far, the most commonly detected genospecies in specimens from patients was *B. afzelii* followed by *B. burgdorferi* sensu stricto, *B. garinii* and *B. bavariensis*.

Performing MLST analysis gives an insight into epidemiology and borrelial population structure as well as into the phylo-geographical and ecological evolution of the different genospecies. Analysing strains obtained from patient specimens reveals the pathogenic potential of certain strains. These findings add valuable information to the international MLST database for future research on Lyme borreliosis.

P63 **Optimized fixation of actin filaments for indirect immunofluorescence staining of rickettsiae**

Monika Danchenko[1], Lucia Csaderova[1], Pierre Edouard Fournier[2], Zuzana Sekeyova[1]*

[1] Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia
[2] UMR VITROME, AMU, IRD SSA, AP-HM, IHU Mediterranee Infection, Marseille, France

**Background** Rickettsiae are arthropod-borne bacteria, recognized as human pathogens. For an accurate diagnosis, in addition to clinical symptoms and molecular identification, immunofluorescence assay is implemented as the "gold standard". Moreover, indirect immunofluorescence is widely used for the detection of pathogens in cell cultures and the investigation of cell structure and function.
**Results** Herein we optimized this serological technique, testing four fixative solutions: 3.7% formaldehyde, 4% paraformaldehyde, 4% paraformaldehyde in the cytoskeletal buffer and 4% paraformaldehyde in PHEM buffer, for the sensitive detection of rickettsial antigens, and preservation of intracellular structures of the host cells, particularly filamentous actin. Rickettsial antigens were presented equally well both with formaldehyde and all paraformaldehyde-based fixations, but only protocol with 4% paraformaldehyde in PHEM buffer allowed accurate imaging of actin filaments.
**Conclusions** Overall, our results suggest that 4% paraformaldehyde in PHEM buffer gives the most accurate image of the actin cytoskeleton and simultaneously allows monitoring of rickettsiae using actin-based motility during infection inside the host cells.

**Acknowledgement / Funding** This study was financially supported by the projects: VEGA 2/0010/19 from the Scientific Grant Agency of Ministry of Education, Science, Research and Sport, as well as SRDA SK-FR-2017-0005 from the Slovak Research and Development Agency, and the French National Research Agency under the program "investissements d'avenir", reference ANR-10-IAHU-03.

P64 **Identification and genetic characterization of the etiological agent of horse piroplasmidosis in Eastern Siberia**

Olga Suntsova, Vera Rar, Oksana Lisak, Elena Doroschenko, Yulia Savinova, Aleksei Paramonov, Olga Chernoivanova, Irina Kozlova

Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia; Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia

Horse piroplasmidosis is a natural focal infection caused by the protozoan hemoparasites of the order Piroplasmida - ***Babesia caballi*** and *Theileria equi*. Cases of horses piroplasmidosis are periodically noted in various regions of Russia, but until now the causative agents of this disease in Eastern Siberia have not been genetically characterized.

We have investigated blood samples from 96 horses for the presence of *Babesia* and *Theileria* DNA using two-round PCR followed by sequencing of the positive samples. *B. caballi* DNA was not detected in any of the studied samples, despite the fact that this pathogen was previously detected in many regions of Russia. *T. equi* DNA was found in blood samples in 38.5% of horses from the Irkutsk region. The studied horses were kept in private stables, farms and at the hippodrome of the city of Irkutsk. Based on the nucleotide sequences analysis of the 18S rRNA gene, the *T. equi* samples from the Irkutsk region belonged to two of the four known genetic groups (A-D), significantly differing in the variable (V4) region of the gene. Most of the samples were assigned to the group B. All *T. equi* sequences from this group were identical to each other and corresponded to sequences found in the blood of horses from various countries: China (KF559357), Korea (HM229407), Mongolia (AB733379), Switzerland (KM046918) and Spain (DQ287951). Group B *Theileria* were also found on the territory of Africa (AB515310, EU642507, etc.). *T. equi* sequences of the group A from the Irkutsk region corresponded to the sequences found in the blood of horses from India (KP995259), or differed from it by single substitutions. It should be noted that *Theileria* belonging to the genetic group A are also found in other regions: Europe (AY150062), USA (JX177670) and South Africa (Z15105).

The reported study was funded by RFBR according to the research project №19-016-00156) a.

P65 **Study of the genetic variability of Babesia in the territory of the Irkutsk region**

Olga Suntsova, Oksana Lisak, Elena Doroschenko, Yulia Savinova, Olga Chernoivanova, Aleksei Paramonov, Irina Kozlova

Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia

The purpose of this work was to study the genetic variability of the *Babesia* genus protozoan hemoparasites in ixodic ticks and small mammals in the Irkutsk region.

During 2013-2017, 1143 specimens of ***Ixodes persulcatus***, 298 of *H. concinna* and 177 of *D. nuttalli* were investigated for presence of *Babesia* DNA. *Babesia* DNA was detected by two-round PCR in the presence of genus-specific primers for the 18S rRNA gene region [Rar W.A. et al. 2005; Fedulina O.O. et al. 2013].

The infection of *I. persulcatus* and *H. concinna* ticks with Babesia in the Ekhirit-Bulagatsky district of the Irkutsk region was 4.2% and 3.0%, respectively. *Babesia* DNA was not detected in *D. nuttalli* ticks. In the Irkutsk district, infection of *I. persulcatus* with *Babesia* was lower (0.8%).

The analysis of the nucleotide sequences of the 18S rRNA gene of *Babesia* found in ixodic ticks was conducted. *Babesia* identified in ixodic ticks in the Ekhirit-Bulagatsky district belonged to the *Babesia* of cattle and small ruminants group, where they formed two clusters. *Babesia*, similar to the *Babesia crassa* sheep pathogen, were attributed to the first cluster (level of similarity 97-99.9%). *Babesia* belonging to this cluster, were found in both *I. persulcatus* and *H. concinna*. The Irk-Hc130 isolate from the *H. concinna* tick from the Ekhirit-Bulagatsky district belonged to the second cluster; it was genetically closest to the *B. motasi* isolated from a sheep in the Netherlands. In the Irkutsk district *B. microti* Us-type and *B. venatorum* DNA was identified in *I. persulcatus* ticks. Nucleotide sequences of the 300 bp long 18S rRNA gene fragment were determined in 6 positive samples of *Babesia* from the liver of the tundra voles caught in the Ekhirit-Bulagatsky district. Analysis of the nucleotide sequences of these samples confirmed their belonging to the *B. microti* US-type. Earlier this pathogenic for human *Babesia* species was identified in *I. persulcatus* ticks in the Irkutsk district.

This study is funded by RFBR research project №19-016-00156) a.

P66 **Babesia crassa – like infection in Slovenia**

Katja Strašek Smrdel and Tatjana Avšič

Institute of Microbiology and Immunology, Faculty of Medicine, Zaloška 4, SI-1000 Ljubljana, Slovenia

Babesiosis is a world-wide disease of animals and humans, specially splenectomized individuals. Since the first case in north - western part of former Yugoslav Republic of Croatia number of reports is increasing. Human infection has been associated with only a few species, mainly *Babesia microti* and *Babesia divergens*. Previously animal pathogens are now being reported also as human pathogens, not only in immunocompromised patients, but also in healthy individuals. Moreover, new species of *Babesia spp*. are emerging. Haemoparasite *B. crassa* was first mentioned as an animal pathogen in Turkey and Iran. Recently, a paper from China describes *B. crassa* - like parasite as a human pathogen, that causes symptomatic and asymptomatic infections. Hereby, we present babesiosis in an immunocompromised Slovenian patient without spleen. The infection was confirmed with bloodsmear examination, with PCR for 18S rRNA gene and, at last, with seroconversion of IgG antibodies. Sequencing of a nearly complete 18S rRNA gene revealed *B. crassa* - like pathogen. We performed additional PCR and sequencing of beta-tubulin gene that confirmed our previous result. In a phylogenetic tree of 18S rRNA sequences from babesiae a sequence of a babesia from our patient clustered together with *B. crassa* – like sequences from ticks from Hungary, Russia and China. This newly recognized *Babesia* genotype has been detected in Far East Asia, Siberia and also in central Europe in Hungary and Slovakia, in questing, rodent- and bird-attached ticks. In Europe, this is the first report of *B. crassa* - like infection in an immunocompromised person without spleen.

P67 **Molecular survey of piroplasms (Babesia spp., Hepatozoon spp.) in micromammals in Romania**

Andra Toma-Naic, Maria Emilia Nedișan, Silvia-Diana Borșan, Vasile Cozma

Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and, Veterinary Medicine, Cluj-Napoca, Calea Mănăștur 3-5, 400372 Cluj-Napoca, România

Piroplasms are small intra-erythrocytic protozoan parasites, which are widely distributed in mammals. These Apicomplexan protozoans include many parasites of medical importance, to livestock and even humans. Babesiosis is an emerging and potentially zoonotic disease caused by tick-borne parasites of the *Babesia* genus. *Hepatozoon spp*. infects all classes of terrestrial vertebrates mostly carnivores (canids, felids, hyaenids, viverrids, mustelids and procyonids). During the past decade molecular tools for characterization and monitoring parasite populations have been firmly established as an integral part of field studies and intervention trials. A study regarding these tick-borne protozoan infections in small mammals was carried out in 15 countys in Romania. This study focused on the molecular identification of tick-borne pathogens (*Babesia* and *Hepatozoon*) in blood samples of 377 micromammals. Out of 377 DNA samples, 30.77% (C.I: 26.3-35.6%) were positive for the presence of blood piroplasms (*Babesia spp* and *Hepatozoon spp*). Of the 9 analyzed orders of small mammals, the highest prevalence was found in voles: 65.80%, 54/82 (C.I: 54.5-75.97%); mice: 27.30%, 40/147 (C.I. 20.2-35.2%) and shrews: 16.10%, 10/62 (C.I.: 8-27.7%), [$p < 0.00001$]. As far as species prevalence is concerned, of the 26 analyzed species of small mammals, the highest prevalence was found in: *Microtus arvalis*: 78.72%, 37/47 (C.I. 65.3-89.3%); *Myodes glareolus*: 62.07%, 18/29 (C.I.:42.3-79.3%); *Apodemus uralensis*: 36.70% , 8/22 (C.I.:17.20-59.3%), [$p < 0.00001$]. As the majority of micromammals live in proximity of domestic (urban and periurban) areas, they could represent potential reservoirs for human and animal piroplasms.

P68 **Genetic diversity of representatives of the species Rickettsia in natural foci of the Irkutsk region (Russia)**

Dzhioev Yu.P.1, Kozlova I.V.2, Tkachev S.E.3, Rar V.A.3, Doroschenko E.K.2, Lisak O.V.2, Suntsova O.V.2, Paramonov A.I.2, Savinova Yu.A.2, Belyaeva A.S.1, Zlobin V.I.1

1Irkutsk State Medical University, Irkutsk, Russia;, 2Scientific Center for Family Health and Human Reproduction Problems, Irkutsk, Russia;, 3 Institute of Chemical Biology and Fundamental Medicine of the SB RAS, Novosibirsk, Russia

In natural foci of tick-borne pathogens in the Irkutsk region, several species of ticks from the *Ixodidae* family have been identified: *I. persulcatus, I. lividus, I. trianguliceps, Dermacentor nuttalli, D. silvarum, Haemophysalis concinna*. Of these, *D. nuttalli* tick is the main vector of *Rickettsia sibirica* and carries a range of diverse genetic variants of *Rickettsia*, potential human pathogens. To identify the degree of genetic diversity of *Rickettsia*, studies were carried out on 2 samples of *D. nuttalli* ticks collected in natural foci of two regions of the Irkutsk region: Olkhonsky (12 ticks) and Ekhirit - Bulagatsky (48 ticks). For genetic typing of *Rickettsia* species, PCR amplification and sequencing methods were used, where the targets were genes: 16S rRNA and gltA (citrate synthase gene). As a result of the sequence, 14 positive *Rickettsia* samples were obtained: the 16S RNA 4 gene and 10 gltA gene. On the phylogenetic tree of the 16S rRNA gene, all 4 samples were included in the *Rickettsia raoultii* cluster, but 1 of them was identical to *R. raoultii* sp. DnS28, and 3 others were typed as *R. raoultii* sp. RpA4. On the tree, the gltA gene samples were also divided into two groups. Of the 10 samples, 2 were closely related to *R. raoultii* sp. DnS28, but separated into a separate subgroup. The other 8 samples formed a cluster with *Candidatus* Rickettsia tasmanensis. This new genetic variant of *Rickettsia* was isolated from *Ixodes tasmani* ticks collected from marsupial mammals (Tasmanian devils - *Sacrophilus harrissi*), inhabited only on the island of Tasmania. *Candidatus* R. tasmanensis is a new candidate for the status of an independent species, which does not yet have a clearly defined genetic relationship with the identified species of rickettsiae. It remains unclear how this new candidate version of *Rickettsia* could be included in the circulation of rickettsiae in the territory of the Irkutsk region.

P69 **Laboratory diagnosis of Human Granulocytic Anaplasmosis in South Korea**

Kyeong Ah Lee, Seung Hun Lee, Hwnhee Kim, Jeong-Hoon Chun, Seon Do Hwang and Kyu Jam Hwang

Division of Bacterial Diseases, Center for Laboratory control of Infectious Diseases, Korea Centers for Disease Control and Prevention, Cheongju, Chungcheongbuk-do 28159, Korea, Division of Zoonoses, Center for Immunology and Pathology, Korea National Institute of Health, Korea, Centers for Disease Control and Prevention, Cheongju, Chungcheongbuk-do 28159, Korea, Yeosu National Quarantine Office, Korea Centers for Disease Control and Prevention, Yeosu, Jeollanam-do 59729, Korea

*Anaplasma phagocytophilum* is an obligate intracellular bacterium that causes an acute febrile illness known as Human granulocytic anaplasmosis (HGA) or anaplasmosis. The organism is a tickborne pathogen transmitted by mainly *Haemaphysalis* spp. or *Ixodes* spp. HGA generally presents with nonspecific symptoms such as fever, headache, malaise, myalgia, thrombocytopenia, and leukopenia. HGA was first identified in United State in 1994. In South Korea, HGA was first reported in 2013. PCR or serologic test for diagnosis of HGA has been conducted at Korea Centers for Disease Control and Prevention (KCDC) since October 2014. This study shows the result of laboratory diagnoses of HGA in suspected patients using immune-fluorescent antibody assay (IFA) and PCR from 2015 to 2018.

Sera and blood samples were collected from the suspected HGA patient, 2015-2018. We have conducted serological tests of HGA by IFA test from the sera of 1987 patients for four years. Nested PCR was performed to amplify 16S rRNA gene of *A. phagocytophilum* of 953 blood samples.

In serological test using IFA for 2015-2018, seropositive rates for IgG or IgM of *A. phagocytophilum* were 6.96% (14/201), 5.30% (16/302), 9.36% (56/598) and 21.22% (188/886), respectively. Seropositive cases in IFA has steadily increased year by year. In 2018, there were 32 patients showing 4 fold increase between initial phase and convalescence phase, and 4 patients in 2015. In South Korea, detection rate of anaplasmosis is relatively low compared with other tick-borne diseases such as severe fever with thrombocytopenia syndrome (SFTS) and Scrub typhus. However, additional attention and disease control regarding anaplasmosis are needed because the number of positive cases in South Korea tend to increase gradually. This work was supported by Korea Centers for Disease Control and Prevention (4800-4837-301-210-13, 4800-4838-303-210-13)

P70 **Detection of persistent forms of Borrelia burgdorferi sensu stricto in infected mice after antibiotic treatment: cultivation of viable spirochetes from the sites of secondary infection**

Natalie Rudenko, Maryna Golovchenko, Ida Ramzy, Hana Sehadova, Marie Vancova

Biology Centre Czech Academy of Sciences, Institute of Parasitology, Institute of Enthomology

Lyme borreliosis is a multisystem disorder caused by the certain species of spirochetes from the *Borrelia burgdorferi* sensu lato (s.l.) complex. Since all *Borrelia* species are host-propagated bacteria that move between a vertebrate host and tick vector, the spirochetes have developed strategies to sense and survive in these diverse environments. Survival of spirochetes in hostile environment is achieved by regulation of differential gene expression in response to changes in temperature, salts, nutrient content, acidity fluctuation, multiple host or vector dependent factors and leads to formation of dormant subpopulations of cells. Alterations in the level of genes expression in response to antibiotic pressure lead to establishment of persisters' subpopulation. “Dormancy” and “persistence” do share some similarities: both represent the cells with low metabolic activity that can exist for extended periods without replication, both constitute the populations with different gene expression profiles and both differ significantly from replicating forms of spirochetes. Persisters are elusive, present in low numbers, morphologically heterogeneous, multidrug-tolerant cells that can change with environment.

We studied the effect of doxycycline, amoxicillin and combined antibiotic treatment on elimination of infection with *Borrelia burgdorferi* senso stricto *ospC* type B strain using the mice model. Antibiotic treatment of infected mice started 4 weeks post-infection (chronic infection) and copied the treatment protocol used for human patients. After the end of antibiotic treatment mice blood, bladder, spleen, heart, brain and joints were collected for immunohistochemical staining followed by electron microscopy analysis and cultivation of spirochetes in MKP media. Viable spirochetes were cultured from bladder, brain and joints of all amoxicillin treated mice and from joints of mice treated with both antibiotics. Immunohistochemistry confirmed the presence of spirochetes in tissues of treated mice. Cells morphology is studied by electron microscopy.

**Acknowledgement** Supported by MEUS (LM2015062Czech-Bioimaging and Z.02.1.01/0.0/0.0/16_013/0001775*)* andMinistry of Health of CR (NV19-05-00191).

P71 **Interaction of Borrelia burgdorferi with cultured tick cells**

Katarína Schwarzová1 and Eva Špitalská2

1Institute of Microbiology, Faculty of Medicine Comenius University and University Hospital, Bratislava, Slovakia and 2Biomedical Research Center, Slovak Academy of Sciences, Bratislava

Adhesion is the initial event in the establishment of any infection. Without the ability to adhere to host cells surface, there is no invasion, dissemination, or persistence and host colonization by many bacterial pathogens. *Borrelia burgdorferi* cells have been shown to adhere to human and murine fibroblasts, endothelial cells, epithelial cells, macrophages, neuronal and glial cells, fibrocytes, and lymphocytes; also tick cells, and Vero cells. At present we don't have enough information such as the infection dynamic processes, intravascular transport of the *Borrelia*, mechanisms of borrelia escape from the vascular network and possible role of cell components involved in organotropism. *B. burgdorferi* cells have been shown to adhere to human and murine fibroblasts, endothelial cells, epithelial cells, macrophages, neuronal and glial cells, fibrocytes, and lymphocytes; also tick cells, and Vero cells.

Spirochetes adhesion and penetration were examined by electronmicroscopy using a tick cell line from *Ixodes ricinus*. The initial phase of the interaction between long-term cultivated. *B. burgdorferi* and the cell lines was studied. Within 30 min after cultivation spirochetes started to bound to the tick cells by one end. A vertical contact between some borreliae and examined cells was confirmed already after one hour of incubation at 37 °C, while the lateral contact was observed as well after extended period of incubation. A cytotoxic effect of borreliae could be observed when the time of incubation was extended to 2 h. Spirochetal round cysts and small granules were also noted in the cultivated strain of *Borrelia burgdorferi*. Whether these morphological forms are involved in adhesion and invasion of the host cells remains to be open for further research.

P72 **Interaction of Borrelia burgdorferi with cultured Vero cells**

Katarina Schwarzova1 and Eva Špitalská2

1Institute of Microbiology, Faculty of Medicine Comenius University and University Hospital, Bratislava, Slovakia and 2Institute of Virology, BMC SAS, Bratislava, Slovakia

*Borrelia burgdorferi* spirochetes are able to persist in the human body and play with its immune system by producing a biofilm-like protein envelope. Thus, the patient's organism does not produce antibodies, the serological results are negative and the infection passes into a chronic stage. Such mechanisms of long-term survival of borreliae in the host are their active invasion of host tissues, spirochetal cells in a metabolically inactive form, or release of toxic substances into the bloodstream following destruction of spirochetal cells. Adhesion is the initial event in the establishment of any infection. Without the ability to adhere to host cells surface, there is no invasion, dissemination, or persistence and host colonization by many bacterial pathogens. *B. burgdorferi* cells have been shown to adhere to human and murine fibroblasts, endothelial cells, epithelial cells, macrophages, neuronal and glial cells, fibrocytes and lymphocytes; also tick cells, and Vero cells. At present we don't have enough information such as the infection dynamic processes, intravascular transport of the *Borrelia*, mechanisms of *Borrelia* escape from the vascular network and the possible role of cell components involved in organotropism.

Spirochetes adhesion and penetration were examined by electronmicroscopy using a tick cell lines from *Ixodes ricinus*. The initial phase of the interaction between long-term cultivated *Borrelia burgdorferi* and the cell lines was studied. Within 30 min after cultivation spirochetes started to bound to the tick cells by one end. A vertical contact between some borreliae and examined cells was confirmed already after one hour of incubation, while the lateral contact was observed as well after extended period of incubation. A cytotoxic effect of borreliae could be observed when the time of incubation was extended to 2 h.

P73 **Reinfection with Borrelia burgdorferi sensu lato – comparison of isolates obtained from erythema migrans**

Eva Ružić-Sabljić1, Vera Maraspin2, Stanka Lotrič-Furlan2, Daša Stupica2, Katarina Ogrinc2, Petra Bogovič2, Tereza Rojko2, Franc Strle2, Tjaša Cerar Kišek1

1Institute of Microbiology and Immunology, Faculty of Medicine Ljubljana, Slovenia 2Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia

**Background** Lyme borreliosis is caused by several *Borrelia* species transmitted via tick bite. Inhabitants living in Lyme borreliosis endemic regions such as Slovenia may undergo more than one *Borrelia* infection in their lives. Reinfection is defined as new infection with *Borrelia* species occurring after successfully treated previous infection. Herein we compare *Borrelia* isolates obtained from 90 patients who had erythema migrans (EM) two times and in whom on both occasions *Borrelia* was isolated from the skin lesion.

**Material and methods** Skin obtained from the border of a typical erythema migrans was inoculated into the MKP medium and incubated at 33 °C. Isolated *Borrelia* strains were identified to species level by MluI restriction of genomic DNA (MluI-restriction fragment length polymorphism, RFLP); strains were delineated within individual species by plasmid profiling.

**Results** From 1992 to 2017 there were 90 patients with culture positive EM reinfection. Of 180 isolates, 161 (89.4%) were *Borrelia afzelii*. In 71/90 (78.9%) patients *B. afzelii* was isolated from the initial as well as from the next EM skin lesion, while in 19/90 (21.1%) patients *Borrelia* species differed but one of the isolates in the individual pair was *B. afzelii*. All *B. afzelii* strains belonged to Mla1 RFLP but their plasmid profiles differed significantly. Of 12 *B. garinii* strains, 11 were Mlg2 and one Mlg1, of six *B. burgdorferi* sensu stricto strains five were Mlb2 and one Mlb8, while *B. spielmanii* was identified as Mls1.

**Conclusions** In Slovenia, *B. afzelii* is the most common species causing EM not only in cases of primary infection but also when early Lyme borreliosis was the result of reinfection. However, in pairs with concordant *Borrelia* species *B. afzelii* strains were heterogeneous according to plasmid profiles and in none of 90 patients reinfection with the same strain was confirmed.

P74 **LC-MS/MS based metabolomics of Borrelia infection in mouse model**

Otto Glader1, Maarit Karonen2 and Jukka Hytönen1, 3

1 Institute of Biomedicine, University of Turku, Turku, Finland, 2 Institute of Chemistry, Natural compound Chemistry, University of Turku, Turku, Finland, 3 Clinical Microbiology, Turku University Hospital, Turku, Finland.

*Borrelia* diagnostics is currently based on clinical evaluation of the disease and laboratory testing. Local infection cases are diagnosed by clinical evaluation, but in disseminated cases laboratory testing is required. Serology is the main method of laboratory testing and is performed by ELISA and immunoblot methods. Current serology detects only 20-50% of disseminated infections. Problematic is also detecting re-infections because of antibody levels which stay high after infection. (1,2)

In this study novel *Borrelia* infection biomarkers were searched for by UHPLC-MS/MS method using serum and urine of infected (20 animals) and control (20 animals) mice as sample material. Differences in small molecular weight compounds between control and infected samples were the interest of this study. Mice were infected with *Borrelia* bacteria and were followed up for 4 weeks. At the end of the experiments, total blood and urine samples were collected.

UHPLC–MS/MS analyses were carried out with an Acquity UHPLC system (Waters Corp) consisting of a sample manager and photon-diode array detector, and a Q-Exactive Orbitrap mass spectrometer (ThermoScientific) with an electrospray ionization interface. Data analysis was performed with Xcalibur software (ThermoScientific).

Small molecule metabolite differences between infected and control mice were observed in both serum and urine samples. Major differences in the concentrations of certain amino acids, immune system energy metabolites and purine synthesis metabolites were detected both in urine and serum samples. Final results and result interpretation will be presented at the conference.

1.Stanek G, Wormser GP, Gray J, Strle F.2012. Lyme borreliosis. Lancet 379:461-73.

2.Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP.2005. Diagnosis of lyme borreliosis. Clin Microbiol Rev 18:484-509.

P75 **Complement-mediated serum susceptibility of Borrelia burgdorferi sensu lato strains from Serbia**

Gorana Veinović, Darko Mihaljica, Ratko Sukara, Sanja Ćakić, and Snežana Tomanović

Institute for Medical Research, University of Belgrade, Dr Subotića 4, POB 102, 11129 Belgrade, Serbia

Lyme borreliosis, the most common tick-borne disease in Eurasia and North America, is caused by spirochetes of *Borrelia burgdorferi* sensu lato complex. *Borrelia* strains exhibit different pathogenicity even within the same species and innate immunity of a host plays an essential role in the recognition, discrimination, and elimination of invading microorganisms. Since host specificity of *Borrelia* is dependent on resistance to host complement, we were interested in susceptibility of different *Borrelia* strains (five *Borrelia lusitaniae*, three *Borrelia garinii*, two *Borrelia afzelii*, two *Borrelia bavariensis*, and two *Borrelia valaisiana*) isolated from *Ixodes ricinus* ticks from Serbia, to human complement.

The direct killing assay was used to investigate the susceptibility of *Borrelia* strains, *in vitro*. Strains were cultured until they reached 10^6 cells/mL. Serum samples were pooled for normal human serum (NHS) while heat-inactivated serum (HIS) was generated by incubating NHS at 56°C for 45 min. The equal volumes of strain culture and NHS or HIS were mixed in microtiter plates and incubated at 33°C for 1 and 3 h. After incubation, samples were examined by dark-field microscopy and 100 *Borrelia* per well were scored as motile or immotile. Loss of motility and extent of blebbing of *Borrelia* in NHS compared to HIS was indicative of complement-mediated killing and inactivation of *Borrelia*.

After 3 h of incubation, 2/2 *B. afzelii*, 2/2 *B. bavariensis*, and 1/3 *B. garinii* strains were motile (98.4-100% motility), 5/5 *B. lusitaniae* and 2/3 *B. garinii* strains were mostly immotile (0-5.2% motility), while two *B. valaisiana* strains were motile (82.5% and 64.3%).

This study is the first report on *in vitro* susceptibility of local *Borrelia* strains to human serum and shows differences in susceptibility among various isolates. Since there is no data on *Borrelia* species infecting humans in Serbia, our results point toward a pathogenic potential of local *Borrelia* strains.

P76 **Co-infection and competition between strains of Borrelia afzelii in immature Ixodes ricinus ticks**

Dolores Genné1, Anouk Sarr1, Olivier Rais2, Maarten J. Voordouw1, 3

1 Laboratory of Ecology and Evolution of Parasites, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland, 2 Laboratory of Ecology and Epidemiology of Parasites, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland 3 Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada

Vector-borne pathogens often consist of genetically distinct strains. These strains can establish mixed infections or co-infections in the arthropod vector. Co-infections can result in competitive interactions between strains with important consequences for strain abundance and transmission. Here we used two different strains of the Lyme disease pathogen, the spirochete bacterium *Borrelia afzelii*, to investigate the interactions between strains inside the tick vector, *Ixodes ricinus*, at different life stages: engorged larvae, 1-month-old nymphs, and 4-month-old nymphs. As the spirochete population inside the nymph declines over time, we expected the strength of competition between strains to increase with nymphal age. We also tested whether temperature influenced inter-strain competition by exposing nymphs to different temperature treatments. Larvae were fed on mice infected with either one or two strains of *B. afzelii*. Engorged larvae were allowed to moult into nymphs and nymphs were either sacrificed (1-month-old nymphs) or kept in three different temperature treatments for 3 months before sacrifice (4-month-old nymphs). We used strain-specific qPCRs to quantify the abundance of each strain in the engorged larvae, 1-month-old nymphs and 4-month-old nymphs. We found that competition between strains of *B. afzelii* occurs at all three life stages; both strains had reduced spirochete loads in ticks that had fed as larvae on co-infected mice. The *B. afzelii* spirochete loads in the 4-month-old nymphs was 41% lower compared to 1-month-old nymphs, indicating that the spirochete population decreases over time, but surprisingly this phenomenon was not influenced by the temperature treatment. We predict that the combination of competition between strains combined with the age-related decline in the spirochete population will eventually result in the loss of nymph-to-host transmission of the low abundance strains.

P77 **Subtypes of Borrelia burgdorferi sensu lato strains from Serbia characterized by pulsed-field gel electrophoresis after MluI restriction of genomic DNA**

Sanja Ćakić 1, Tjaša Cerar-Kišek 2, Gorana Veinović1, Darko Mihaljica 1, Ratko Sukara 1, Eva Ružić-Sabljić 2 Snežana Tomanović1

1Group for Medical Entomology, Centre of Excellence for Food and Vector Borne Zoonoses, Institute for Medical Research, University of Belgrade, Belgrade, Serbia, 2Laboratory for Diagnostics of Borreliosis and Leptospirosis, Institute for Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

The geographic distribution of *Borrelia burgdorferi* sensu lato species in Europe exhibits dynamic spatial and temporal variations. The observation that genetically divergent strains within the same *Borrelia* species show different tendencies for haematogenous dissemination after tick bite gave rise to the notion of different pathogenicity of strains. The aim of this study was subtype delineation of *B. burgdorferi* sensu lato strains isolated from unfed *Ixodes ricinus* ticks from different eco-geographical regions in Serbia. It has been shown that pulsed-field gel electrophoresis after *Mlu*I restriction of the genomic DNA (*Mlu*I Large Restriction Fragment patterns- MluI-LRFP) represents a highly specific and reproducible method for *Borrelia* genotypization. Results of the present study are based on 28 local strains previously classified into four species: *B. afzelii* (n=14), *B. garinii* (n=6), *B. valaisiana* (n=2), *B. lusitaniae* (n=8). Using MluI-LRFP, we were able to delineate all *Borrelia* species included in the study. Each of the 4 examined *Borrelia* species displayed unique MluI-LRFPs that enabled straightforward separation of strains into particular species, and also subtypes of strains within species. Among analyzed strains following MluI-LRFP subtypes were recognized: *B. afzelii* - Mla1 (13/14, 92.8%) and Mla2 (1/14, 7.2%), *B. garinii* - Mlg1 (1/6, 16.7%) and Mlg2 (5/6, 83.3%), *B. valaisiana* - Mlv1 and Mlv2, *B. lusitaniae* - Mll2 (2/8, 25%), Mll3 (2/8, 25%), Mll4 (2/8, 25%), Mll5 (2/8, 25%). The subtypes of *B. lusitaniae* (Mll3, Mll4, and Mll5) identified in the present analysis have not been reported previously. Considering the presence of different subtypes of pathogenic species, *B. afzelii*, *B. garinii*, and two species with a potential pathogenic risk, i.e. *B. lusitaniae* and *B. valaisiana*, we conclude that Serbia represents an area with a high risk for Lyme borreliosis (LB). Genotyping of local strains will greatly improve understanding of LB in Serbia.

P78 **Cruising for bruising?**

Ulrich Schwab, MD, FRCP, FRCPE, DTM&H, Dip GUM, PGCME

Department of Infectious Diseases and Tropical Medicine, Newcastle upon Tyne, UK

A 83 year old man presents with 3 day history of malaise, anorexia, rigors and a dry cough to a peripheral hospital following a cruise along the Scandinavian coastline (Copenhagen, Oslo, Stavanger, Bergen) and the British Isles (Shetland, Orkney). He is febrile at 38.7C, tachycardic but haemodynamically stable, with a marked thrombocytopenia (41), abnormal PT (15), renal impairment (Creatinine 199) raised CRP 117 but normal white cells including differential and CXR. Throat swab, serial blood cultures, and MSU were culture-negative. Despite receiving IV fluids, broad spectrum antibiotics for a presumed lower respiratory tract infection with AKI and delirium his pyrexia persists, his CRP rises to 223, and he develops abdominal pain with jaundice (Bilirubin 158, AST 249, ALT 94, AlkP 277), worsening renal function (Crea 543) oliguria, anaemia (Hb 7.6, haptoglobin <0.03g/l) with worsening confusion and bruising suggestive of a hepato-renal syndrome with DIC prompting transfer to the regional ID unit. PMH includes AVR/TAVI, PUD, Waldenstroem's macroglobulinaemia previously receiving IVIG's and Rituximab, last 3 months prior. Extensive travel history: Nigeria ('60ties,'90 and 2003), Kenya, Malaysia/Taiwan, Indian Subcontinent ('70ties), South Africa ('97), Korea (2011). A blood film suggests malaria 20-30%parasitaemia, posing speciation difficulty. Of note he spent most summers on the Great pond in Belgrade (Maine/USA), most recently 4 weeks before presentation, pointing towards epidemiological Babesiosis exposure particularly in an immunocompromised individual with a negative RDT for *P. falciparum*, later confirmed as 22% parasitaemia, *B. microti* ELISA+ve, IFAT+ve 1:320 (negative blood-borne-virus-screen, *Leptospira*-DNA, Hantavirus-IF, *B. burgdorferi* and *Anaplasma*-IF). Despite IV cefotaxime, artesunate, po doxycycline, atovaquone/azithromycin together with IV clindamycin/quinine, - a pan-reactive auto-Ab panel, prevented exchange transfusion, he deteriorates rapidly despite full organ support and dies from multi-organ-failure. Ferritin elevation >16.000 suggests haemophagocytic lymphohistiocytosis confirmed on post-mortem. Epidemiology, diagnosis, prognosis and treatment of Babesiosis will be discussed.

P79 **Molecular detection of vector-borne pathogens in keds and ticks from deer from the Krkonoše Mountains National Park in the Czech Republic**

Kateřina Kybicová1, Pavla Balátová1, Alena Lukavská1, Jiří Navrátil1, Jiří Lamka2

1 National Institute of Public Health, National Reference Laboratory of Lyme Borreliosis, Prague, Czech Republic, 2 Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

Vector-borne infections are very widespread in Europe. The aim of this study was to assess the prevalence of *Anaplasma, Rickettsia, Bartonella, Babesia* and *Borrelia* among ectoparasites hard ticks and keds from deer from the Krkonoše Mountains National Park.

A total of 363 samples of ectoparazites, 139 ticks *Ixodes ricinus* (76 fed female, 29 unfed female, 34 male) and 224 keds *Lipoptena* spp. from 121 red deer and roe deer were investigated during the year 2014-2016. DNA was isolated from whole ticks or keds. All the 363 samples were screened for pathogens by nested PCR and by real-time PCR with assay for *ospA*, *msp2*, *gltA*, 18S rRNA. All positive samples were identified by direct sequence analysis.

Ticks collected from red deer and roe deer were highly infected by *A. phagocytophilum* (50 ticks, 36%) and *Rickettsia* spp. (23 ticks, 16.5%). Only one tick and two ticks were positive for *B. afzelii* and *Babesia* spp., respectively. We found a comparatively lower level of infection of *A. phagocytophilum* (17 keds, 7.6%) and *Rickettsia* spp. (4 keds, 1.8%) in keds. The highest percentage of *Bartonella* infection we detected in keds (63 keds, 28.1%). Four keds (1.8%) was infected by *Babesia* spp. We found no *Borrelia* infection in keds.

Keds from deer were highly infected by *Bartonella* spp. and also infected by *A. phogocytophilum*. Our study shows that keds from roe deer and red deer might be involved in the natural transmission cycle of these vector-borne pathogens. Ticks from deer were highly infected by *A. phagocytophilum* and *Rickettsia* spp.; ticks and deer might play an important role in maintenance cycle of *Rickettsiaceae.*

*Acknowledgement:* This study was supported by MH CZ - DRO (The National Institute of Public Health—NIPH, 75010330).

P80 **The borrelial myocarditis in dog - 1st case report**

Pavel Schánilec, Carlos F. Agudelo, Kateřina Kybicová, Jana Doležalová, Nikola Pantchev, Jiří Nepeřený, Mišo Škorič

Small Animal Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, The Czech Republic, National Reference Laboratory for Lyma Borreliosis, The National Institute of Public Health, Praha, The Czech Republic, Clinic Laboratory for Small Animals, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, The Czech Republic, Department of Infectious Diseases, Idexx Laboratories, Westbrook, United States, Bioveta a.s., Ivanovice na Hané, Department of Pathological Morphology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno

Borreliosis is a well-known condition that can induce several clinical syndromes in human beings and animals leading to several complications even death. The less common myocardial form of the disease can lead to arrhythmia and myocardial inflammation; those changes may induce cardiomyopathy, which is a serious manifestation of the disease though rare.

This presentation describes a case of an 8-year-old female Labrador retriever that was admitted due to progressive movement complications (reluctance to move, fatigue), neuromuscular signs (generalized body weakness, decreased flexor reflexes, dysphonia) and polypnea and irregular heart rhythm. The female was often infested by ticks.

During hospitalization the patient developed different types of arrhythmias (supraventricular tachycardia, several degrees of atrioventricular blocks and ventricular bigeminism) that did not respond well to therapy (infusion therapy, doxycycline, antiarrhythmics, furosemide). There was seropositivity to *Borrelia* sp. (positive *Borrelia*-C6 SNAP 4Dx Plus test and *Borrelia* indirect fluorescent antibody IgM and IgG tests confirmed with Western blott with positive VlsE and OpsC bands). Negative ELISA IgG OspA confirmed that animal was not vaccinated against *Borrelia* sp. Serum was also positive for *Toxoplasma gondii* IgM and IgG antibodies.

Increased cardiac troponins and NT-proBNP showed severe myocardial damage and ongoing cardiac failure. The dog subsequently died as a result of heart disease and arrest after ventricular fibrillation. Pathology demonstrated myocarditis.

Real-time PCR of myocardium confirmed positivity to borreliosis. Positive sample was sequenced as *B. burgdorferi* sensu stricto. DNA *T. gondii* was not detected in heart tissue samples by real-time PCR.

The correlation of serology, cardiac markers, clinical course, pathology concluded a Lyme myocarditis. The myocardial damage caused by *T. gondii* was excluded negative PCR.

To the authors' knowledge this would be the first confirmed case of borreliosis inducing myocarditis and sudden death in a dog.

P81 **Study of animal-attached ticks for Rickettsiae spp. presence and first evidence of Siberian Rickettsia species in Europe**

Maria Vikentjeva 1, 2, Julia Geller 1, Jaanus Remm 3, Irina Golovljova 1

1 Department of Virology and Immunology, National Institute for Health Development, Tallinn, Estonia;, 2 Department of Gene Technology, Tallinn University of Technology, Tallinn, Estonia;, 3 Department of Zoology, University of Tartu, Tartu, Estonia

*Rickettsia* SFG species are tick-associated bacteria which can be transmitted transovarially and transstadially by *Ixodidae* family ticks. There are three *Ixodidae* tick species confirmed to circulate in Estonia: two non-nidicolous generalists – *Ixodes ricinus* and *I. persulcatus*, and nest-dwelling species *I. trianguliceps*. Territorial sharing between various tick species also provides opportunities for tick-associated pathogens to co-circulate within natural environment conditions.

Our previous studies had shown the presence of three *Rickettsia* spp. in unfed questing *I. ricinus* and *I. persulcatus*: *Rickettsia helvetica*, *R. monacensis* and *Candidatus* R. tarasevichiae. Although there is still no clear evidence of *Rickettsia* spp. natural hosts, recent studies converge that besides *Ixodes* ticks rodents could be the potential reservoir hosts for *R. helvetica*.

This study aimed to detect and characterize *Rickettsia* species in animal-attached ticks. Small mammals were caught in five study sites in Estonia – Järvamaa, Pärnumaa, Lääne-Virumaa, Tartumaa and Saaremaa. Nymphs and larvae of *I. ricinus* (n = 1004), *I. persulcatus* (n = 75) and *I. trianguliceps* (n = 117) fed on small mammals were studied for the presence of *Rickettsia* spp. by nested PCR based on partial *gltA* gene fragment. Ticks were removed from 314 rodents of 5 species (bank voles *Myodes glareolus*, yellow necked mice *Apodemus flavicollis*, striped field mouse *A. agrarius*, common shrew *Sorex araneus* and pine vole *Microtus subterranius*).

Rickettsial DNA was detected in 8,7% (103/1186) of all studied ticks. *R. helvetica* is still most prevalent species in Estonia (97,1%, 100/103). Also, newly described Siberian species *Ca.* R. uralica was found in 2,9% of *I. trianguliceps* ticks.

P82 **Molecular investigation of pathogenic and symbiotic bacteria in African hard ticks infesting wild and domestic animals**

Emanuela Olivieri a, Anna Maria Floriano a, Yohannes Mulatu Tafesse a, Alessandra Cafiso b, Edward Kariuki c, Sumer Alali d, Matteo Montagna d, Davide Sassera a

a Dipartimento di Biologia e Biotecnologie, Università di Pavia, Italy; b Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Italy; c Department of veterinary service, Kenya Wildlife Service, Kenya; d Department of Environmental Science and Policy, Università degli Studi di Milano, Italy

In Africa, the most prevalent tick-borne pathogens are considered to be those belonging to the genera *Rickettsia*, *Anaplasma*, *Ehrlichia*, *Babesia*, and *Theileria*. Very few data on ticks and tick-borne diseases in Africa are found in the literature, notwithstanding their great economic, veterinary and medical impact. Considering that tick symbiotic bacteria can also play important roles in host biology and vector efficiency, we investigated the co-presence of pathogenic and symbiotic bacteria in hard ticks collected from wild and domestic animals from different ecological areas in Kenya, Egypt, and Ethiopia.

Taxonomic identification of ticks was performed by means of both morphological (light microscopy) and molecular (12S rRNA PCR) techniques. Tick DNAs were then screened for the presence of pathogenic and symbiotic microorganisms through ad-hoc molecular approaches (PCR). Overall, 358 hard ticks were taxonomically identified as belonging to *Ixodes*, *Amblyomma*, *Hyalomma*, *Rhipicephalus*, *Dermacentor*, and *Haemaphysalis* genera. Pathogen screening provided information on pathogens circulation in Africa, confirming the presence of *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Borrelia garinii*, *Babesia* spp. *Theileria* spp and *Coxiella burnetii*. More novel is the detection of *Candidatus* Cryptoplasma, a recently described α-Proteobacterium belonging to the family *Anaplasmataceae*, for which little is known regarding pathogenic role, ecology and epidemiology. Furthermore, our work provides insights on the African scenario of tick-symbiont associations showing that *Coxiella* endosymbionts are the most prevalent microorganisms, being detected in sixteen different tick species. An additional endosymbiont, *Midichloria*, was also found in 9 different tick species.

Gene sequences were obtained for all PCR-detected microorganisms, and subsequent phylogenetic analyses are ongoing. Statistical analyses will also be performed, to evaluate patterns of distribution of microorganisms on different hosts and ecological areas, as well as potential co-occurrence and competition between pathogens and symbionts.

P83 **Lyme borreliosis spirochaetes in red foxes (Vulpes vulpes) from Serbia**

Snežana Tomanović1, Ratko Sukara1, Salem Juwaid2, Gorana Veinović1, Darko Mihaljica 1, Aleksadra Penezić2, Duško Ćirović2

1 Group for Medical Entomology, Institute for Medical Research, University of Belgrade, Belgrade, Serbia, 2 Faculty of Biology, University of Belgrade, Belgrade, Serbia

The first human case of Lyme borreliosis (LB) in Serbia was recorded in 1987. Since then, it has been registered with annual incidences of 5.61-13.67/100.000 inhabitans. Previous studies revealed high diversity of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks from Serbia, with *B. lusitaniae* found to be the most dominant species, followed by *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. valaisiana* and *B. bavariensis*. However, except from isolation of two *Borrelia* strains from yellow-necked mouse (*Apodemus flavicollis*), there is no data on vertebrate reservoirs involved in enzootic cycles of LB spirochaetes in Serbia. It has been previously documented that red foxes (*Vulpes vulpes*) may serve as competent reservoirs for *B. burgdorferi* s. l. in Europe. Distribution range of foxes covers the whole territory of Serbia. Foxes are the most abundant medium-sized canids, often present in the vicinity of human settlements and domestic animals. Moreover, they are frequently infested with various tick species. All this impose the need to explore the role of foxes in enzootic cycles of borrelia in Serbia. In total, 129 legally hunted red foxes from the 14 localities in Serbia, were included in the study. Spleen samples were collected over a period of seven years (2010-2016). Conventional PCR and sequencing were used for the detection and characterization of *B. burgdorferi* s.l. Presence of DNA of *Borrelia* was detected in 7 samples (5.4%) originated from 2/73 (2.7%) male and 5/56 (8.9%) female animals collected from 3 localities. Sequencing of 5S-23S rRNA intergenic spacer confirmed three species of *B. burgdorferi* s. l., namely: *B. burgdorferi* s. s., *B. lusitaniae* and *B. garinii.* Prevalence detected in our study (5.4%) is in the range of previously published data for Europe, however, it is the first record of *Borrelia* spirochetes in spleen samples of red foxes.

P84 **Rickettsiae and haemosporidians in migratory and non-migratory birds in Slovakia**

Eva Špitalská1, Eva Boldišová1, Markéta Derdáková2, Alžbeta Vašková2, Radovan Václav2

1Institute of Virology, Biomedical Research Center SAS, Bratislava, Slovak Republic, 2Institute of Zoology SAS, Bratislava, Slovak Republic

Birds serve as maintenance hosts for tick larvae and nymphs, introducing and maintaining tick-borne pathogens. Birds play a role as reservoirs of some species of *B. burgdorferi* sensu lato. The competence of birds as reservoirs for *Rickettsia* is still unclear. Avian haemosporidians *Haemoproteus* sp., *Plasmodium* sp. and *Leucocytozoon* sp. belong to the order Haemosporida, family Haemoproteidae, Plasmodiidae, Leucocytozoidae, respectively, infecting avian hosts and in their life cycle requiring blood-sucking insects (Diptera).

The aim of the study was to reveal the presence, prevalence, diversity and host-associations of rickettsiae and haemosporidians in birds trapped in Drienovská mokraď. After ringing, sex and age of trapped birds was determined. A small sample of blood was taken and stored in 70% ethanol. The presence of *Rickettsia helvetica* was confirmed using TaqMan PCR targeting the 23S rRNA gene (Boretti et al. 2009). The presence of haemosporidian parasites was examined by nested PCRs and SybrGreen PCRs targeting mtRNA gene (Bell et al. 2015). Together 502 birds belonging to 40 species were caught in 2017 year. The most abundant species were *Sylvia atricapilla* (22%), *Erithacus rubecula* (16%) and *Parus caeruleus* (8%). *R. helvetica* was confirmed in 31 (6.2%) samples. Using a nested PCR protocol (Hellgren et al. 2004), haemosporidiae were detected in 30 (20.4%, n=147) samples. Parasites of genera *Haemoproteus* and *Plasmodium* were present in 29 birds, *Leucocytozoon* in 17 birds, and 16 birds were coinfected with haemosporidians of multiple genera. SybrGreen PCR assays showed parasite intensity of 4E+2-4E+6 copies within samples. Upcoming analyses will be done to determine lineages of haemosporidiae and ecological correlates of occurrence of rickettsiae and haemosporidiae.

References

Boretti FS et al. Appl Environ Microbiol 2009, 75:3230–3237.
Bell JA et al. Parasites Vectors 2015, 8:383.
Hellgren O et al. J. Parasitol. 2004, 4:797-802.

*Acknowledgements:* The study was supported by projects APVV-16-0463and VEGA 2/0068/17.

P85 **Survey of tick-borne severe fever with thrombocytopenia syndrome (SFTS) Phlebovirus in animals**

Sun-Woo Han (A), Yoon-Kyoung Cho (A), Jun-Gu Kang (A), Kyoung-Seong Choi (B) and Joon-Seok Chae (A*)

(A) Laboratory of Veterinary Internal Medicine, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Republic of Korea., (B) Department of Animal Science and Biotechnology, College of Ecology and Environmental Science, Kyungpook National University, Sangju, Gyungsangbuk-do 37224, Republic of Korea.

Severe fever with thrombocytopenia syndrome virus (SFTSV) is a novel tick-borne Phlebovirus in the family of Bunyaviridae and a causative agent of an emerging infectious disease in China, Japan, the Republic of Korea (ROK) and Vietnam. SFTS is mainly characterized by fever, leukopenia and thrombocytopenia in human. The purpose of this study is to investigate the host or reservoirs of SFTSV in companion, domestic and wild animals in the ROK.

To investigate the prevalence of SFTSV in the ROK, animal sera were collected from domestic, wild and companion animals during 2013-2019. The aim of this study was to investigate the survey of SFTSV antigen in animals throughout the ROK. Sera were collected from total 2,107 animals in the ROK.

Viral RNA was extracted from sera using viral RNA extraction kit. One-step RT-nested PCR was performed to amplify the S segment of the SFTS virus. The sequence data were analyzed using Chromas and were aligned using CLUSTAL X. The phylogenetic analysis was constructed using the neighbour-joining method in MEGA7.

Three of 264 (1.1%) dogs, 33 of 354 (9.32%) cats, 32 of 1,005 (3.2%) goats, 4 of 240 (1.7%) domesticated pigs, 12 of 99 (12.1%) cattle, 2 of 70 (2.9%) horses, 1 of 21 (4.8%) Korean water deer, and 2 of 54 (3.7%) wild boars were positive for SFTSV. Based on phylogenetic analysis, SFTSV is generally classified into Japanese and Chinese clades.

These results are indicates that SFTS Phlebovirus may infected in several animal species, companion, domestic and wild animals in the ROK.

P86 **First detection and molecular identification of the zoonotic Anaplasma capra in captive deer in France**

Maggy Jouglin, Barbara Blanc, Nathalie de la Cotte, Suzanne Bastian, Katia Ortiz, Laurence Malandrin

BIOEPAR, INRA, Oniris, Nantes, France, Muséum National d'Histoire Naturelle, Réserve Zoologique de la Haute Touche, Obterre, France

Cervids are known to be reservoirs of zoonotic bacteria transmitted by ticks. This study aimed to identify the *Anaplasma* species carried by captive red deer and swamp deer in a wild fauna reserve in France. Blood from 59 red deer and 7 swamp deer was collected and analyzed over a period of two years. A semi-nested PCR targeting the 23S rRNA was performed to detect and characterize *Anaplasma* spp. and determine the presence of zoonotic species. *Anaplasma phagocytophilum* was identified in 14/59 red deer (23.7%) but it was not identified in any of the swamp deer (7 animals). Three sequences could not be assigned to any particular species based on the 23S rRNA sequences. Complementary nested PCR targeting 16S rRNA, *gltA* and *groEL* genes and sequencing analysis then identified these sequences as a recently reported zoonotic species, *Anaplasma capra.* This species was found in 2 red deer (*Cervus elaphus*) and 1 swamp deer (*Rucervus duvaucelii*). This is the first report of the tick-borne zoonotic bacterium *A. capra* in France, a species otherwise described only in Asia (China, Japan, Malaysia and South Korea) in goats, sheep, deer, cattle and Japanese serows (*Capricornis crispus*). The vector of *A. capra* has not yet been identified, even if *A. capra* has been detected in Asia in ticks of the genera *Haemaphysalis* (*H. longicornis*, *H. qinghaienesis*), *Rhipicephalus* (*R. microplus*) and *Ixodes* (*I. persulcatus*). While this bacterium may have been introduced into the reserve by infected imported animals, its local epidemiological cycle via tick transmission seems possible as locally born deer were found infected. Diagnostic methods, especially molecular ones, should take into account the potential infection of animals and humans with this species.

P87 **Epidemiology of Lyme borreliosis in Scotland**

Roger Evans1, Sally Mavin1, Chin Lim1, Alison Smith-Palmer2, Lynda Browning2, Gillian Hawkins2, Dominic Mellor2, Eisin McDonald2

1 Scottish Lyme Disease and other Tickborne Infections Reference Laboratory (SMiRL) Inverness Lyme disease and other tick-borne infections, Microbiology department, Raigmore Hospital, Inverness, IV2 3UJ 2 Health Protection Scotland, Meridian Court, 5 Cadogan Street, Glasgow G2 6QE

Lyme borreliosis is recognised as an infection of public health importance in Scotland. In 2018, there were 238 laboratory confirmed cases of Lyme borreliosis, equating to an incidence of 4.4 per 100,000, which was the highest in the UK. In 2018, males accounted for 64% of cases with the highest incidence in those 55 years and older.

Since 2009, to achieve a better understanding of the epidemiology of Lyme borreliosis, questionnaires have been sent by the Scottish Lyme Disease and other Tickborne Infections Reference Laboratory (SLDTRL) to the clinician managing laboratory confirmed cases; capturing information on clinical presentation, history of tick bite, occupation, where the tick bite was most likely to have occurred and treatment. Among cases with information on clinical presentation, 40% were reported to have erythema migrans (alone or in combination with other symptoms), while 30% were reported to have neurological symptoms either alone or in combination with other symptoms. For 27% of cases, Lyme borreliosis had not been considered a likely diagnosis prior to laboratory confirmation, with reasons cited including no history of a tick bite or no history of erythema migrans.

It is recognised that these laboratory confirmed cases represent just a fraction of the true incidence and may not be representative of those treated without laboratory diagnosis as the current UK guidance is to treat erythema migrans on clinical suspicion rather than seek laboratory confirmation and consider starting treatment in patients without erythema migrans but with high clinical suspicion while waiting for laboratory confirmation.

Work is ongoing in Scotland to increase awareness among clinicians and pharmacists of the importance of early diagnosis and treatment, and also among the public to increase tick awareness including the launch of a website to promote the safe enjoyment of the countryside https://www.nhsinform.scot/bugs-and-germs

P88 **Human seroprevalence of antibodies to tick-borne microbes in southern Norway**

Erik Thomas Thortveit, Audun Aase, Lizette Balle Petersen, Åslaug Rudjord Lorentzen, Åse Mygland, Unn Ljøstad

Department of Neurology, Sørlandet Hospital Trust, Kristiansand, Norway, Department of Infectious Disease Immunology, Norwegian Institute of Public Health, Oslo, Norway, The Norwegian National Advisory Unit on Tick-Borne Diseases, Sørlandet Hospital Trust, Arendal, Norway, Department of Clinical Medicine, University of Bergen, Bergen, Norway, Department of Habilitation, Sørlandet Hospital Trust, Kristiansand, Norway

The tick *Ixodes ricinus* is widespread along the coastline of southern Norway, but data on human exposure to tick-borne microbes are scarce. We aimed to assess the seroprevalence of IgG antibodies to various tick-borne microbes in the general adult population living in a Norwegian municipality with high prevalence of ticks.

All individuals aged 18-69 years with residential address in Søgne municipality (n = 7424) were invited to give a blood sample and answer a questionnaire. Søgne is a coastline municipality in the southernmost part of Norway, and has a high prevalence of ticks. Blood samples from 3568 individuals were available for analysis. All samples were analyzed for IgG antibodies to *Borrelia burgdorferi* sensu lato (Bb), and around 1500 samples for IgG antibodies to other tick-borne microbes. Enzyme-linked immunosorbent assays were applied for detection of antibodies to Bb and Tick-borne encephalitis virus (TBEV), and indirect immunofluorescent assays for detection of antibodies to the other tick-borne microbes.

Serum IgG antibodies to Bb was present in 22.0% (785/3568), TBEV in 3.1% (45/1453), *Anaplasma phagocytophilum* in 11.0% (159/1452), *Babesia microti* in 2.1% (33/1537), *Bartonella henselae/quintana* in 0.1% (2/1451) and *Rickettsia helvetica/conorii* in 4.2% (60/1445). Among individuals not vaccinated against TBEV and/or yellow fever, the seroprevalence of IgG antibodies to TBEV was only 1.4% (6/419). The seroprevalence of IgG antibodies to Bb was significantly higher among males than females (27.7% vs 17.0%, $p < 0.001$), and tended for both genders to increase with increasing age. Serum IgG antibodies to *Anaplasma phagocytophilum* and *Rickettsia helvetica/conorii* was significantly more prevalent ($p = 0.010$ and $p = 0.016$, respectively) among individuals with serum IgG antibodies to Bb than among individuals without.

P89 **Historical perspective on the seroprevalence of Lyme borreliosis in Finland**

Julia Cuellar, Timothée Dub, Jussi Sane and Jukka Hytönen

Institute of Biomedicine, Faculty of Medicine, University of Turku, Turku, Finland.Turku Doctoral Programme of Molecular Medicine, Faculty of Medicine, University of Turku, Turku, Finland., European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden., Department of Health Security, National Institute for Health and Welfare, Helsinki, Finland., Laboratory Division, Clinical Microbiology, Turku University Hospital, Turku, Finland.

**Background** Lyme borreliosis (LB) caused by *Borrelia burgdorferi* sensu lato is the most common vector-borne disease in Europe. Serology is the golden standard to confirm LB diagnosis by laboratory tests. Serology is also used to study the prevalence of LB in a defined population cohort. There have been no studies on the seroprevalence of LB before the description of the multi-systemic disease in the late 1970s.

**Methods** In this study, we used a subset of serum samples of the Finnish Mobile Clinic Health Survey (FMC) collected in the years 1966 to 1972 in Finland. The FMC was a cross-sectional health survey including data of a wide-ranging questionnaire, health examination, X-ray photography, resting electrocardiogram, serum and plasma samples of over 50,000 Finnish participants aged 15 years or older.

The serum samples (n=994) used in this study were obtained from 546 men and 448 women aged 15 to 86 years from 24 locations in different parts of Finland in the years 1968 to 1972.

All serum samples were screened for IgG antibodies by an in-house *Borrelia* whole-cell sonicate enzyme linked immunoassay (ELISA). The screening-positive serum samples were further analyzed by C6 Lyme ELISA kit (Immunetics) and by a second confirmatory test recomBead IgG 2.0 (Mikrogen). The data was analyzed statistically with SPSS Statistics version 22.0 (IBM Corporation).

**Results** The preliminary results suggest that LB seroprevalence in Finland was considerably higher in 1970s compared to the current seroprevalence of 3.9% (regional range 0.87%–6.12%) (van Beek *et al*., 2017). The final results will be presented in the ITPD 2019 -conference.

P90 **Epidemiology of Lyme borreliosis through two surveillance systems: general practitioners and hospitalizations, France, 2005-2017**

Alexandra Septfons1, Lucie Fournier2, Benoit Jaulhac3, 4, Henriette de Valk1, Thierry Blanchon2, Julie Figoni1

1 Santé publique France, Paris, France, 2 Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique IPLESP, AP-HP, Hôpital Saint Antoine, Paris, France 3 Early Bacterial Virulence: Lyme borreliosis Group, Université de Strasbourg, CHRU Strasbourg, Fédération de Médecine Translationnelle de Strasbourg, VBP EA 7290, Strasbourg, France 4 Centre National de Reference des Borrelia, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

Lyme borreliosis (LB) is the most frequent vector-borne disease in mainland France. Its surveillance is based on a sentinel network of general practitioners (SGPs) and the national hospitalization database. We describe surveillance data in order to estimate incidence, detect trends and identify risk groups and high-incidence regions.

SGPs report new diagnoses of LB. Reported cases are validated applying the European Union Concerted Action on LB case definitions. A hospitalized LB case was a person hospitalized with a LB specific diagnosis (ICD10 codes: M01.2 or L90.4 or A69.2 in the absence of any other diagnosis or associated with code(s) compatible with LB symptoms (neurological, cardiac, articular, ocular disorders).

From 2011 to 2017, the mean yearly incidence rate of patients consulting a GP for LB was 55 cases per 100,000 inhabitants ranging from 41 in 2011 to 84 per 100 000 in 2016. Except for 2016, no significant increase in LB incidence was observed over this period. The hospitalization incidence rate (HIR) ranged from 1.1 cases per 100,000 inhabitants in 2005 to 1.5 in 2011 with no significant trend. Significant inter-regional variations in LB incidence and HIR were observed. HIR peaked in 5-9 and 70-79 years old. The incidence rate of LB diagnosed at GP level peaked in 60-69 years old. Erythema Migrans (EM) affected 95% of the patients at primary care level. Among hospitalized cases, the most common manifestation was neuroborreliosis (51%).

These complementary systems allow monitoring of two key indicators: EM and neuroborreliosis and provide a more comprehensive understanding of the epidemiology of LB allowing the analysis of geographical distribution and potential expansion across the country. Public health strategies with a particular focus on the high-incidence age groups and regions should continue to promote preventive measures including checks for ticks after exposure, their prompt removal and self-surveillance of bitten people.

P91 **Serological study Lyme disease for high-risk group of tick-borne diseases in South Korea**

Mi yeon Kim1, Hokyung Oh1, Kwan Lee2, Ji-Hyuk Park2, Kyu Jam Hwang1, Jeong-Hoon Chun1, Seon Do Hwang1

1Division of Bacterial Diseases, Center for Laboratory control of Infectious Diseases, Korea Centers for Disease Control and Prevention, Cheongju, Chungcheongbuk-do 28159, Korea, 2Department of Preventive Medicine, Dongguk University College of Medicine, Gyeongju, Gyeongsangbuk-do 38066, Korea

Lyme disease is caused by a group of related spirochaetes *Borrelia burgdorferi* sensu lato that are transmitted by ticks of *Ixodes ricinuss* complex. Lyme disease is the most common tick-borne infectious disease in North America and in Eurasia countries with moderate climates. In Korea, the Centers for Disease Control and Prevention (KCDC) adopted Lyme disease as a national notifiable disease in 2010. The first confirmed patient case for Lyme disease in Korea was in 2012. From 2015 to 2018, we have investigated the infection rate of Lyme disease for high-risk group of tick-borne diseases with Dongguk University.

From 2015 to 2018, a total of 2,182 samples of forestry workers were tested for Lyme disease. The investigatedforestry workers were consisted of 3 groups which were national forestry workers, Korean national park service workers and civil owned forestry workers. Laboratory diagnosis of Lyme disease was performed with two-tier serological testing in which the first tier was immunofluorescence antibody assay (IFA) and second tier was westernblot. When the result was positive in IFA, westernblot was also performed.

From 2015 to 2018, the seroprevalence of Lyme disease were 0.3%, 0%, 0.3% and seroreactivity were 5.6%, 7.9%, 6.5% respectively for three years.

Tick-borne diseases such as Scrub typhus and Lyme disease are tend to increase, and new tick-borne diseases have been reported continuously in Korea. However, survey on the actual status of tick-borne infectious diseases in Korea is lacking. This study is meaningful in that we investigated forestry workers who were known as a high-risk group of tick-borne infectious diseases. Although the seroprevalence for Lyme disease is low, continuous surveillance are also needed to understand the status and to improve control and prevention of these diseases in high-risk population.

This study was supported by the Korean Centers for disease Control and Prevention (4800-4861-304-260-01, 4800-4838-303-210-13).

P92 **Pathogen prevalence in ticks parasitizing humans; a citizens science application**

Rahel Ackermann-Gäumann 1, 2; Werner Tischhauser 3; Gilbert Greub 2, 4; Marie-Lise Tritten 2, 5; Reto Lienhard 2, 5

1 Federal Office for Civil Protection, Spiez Laboratory, Spiez, Switzerland, 2 Swiss National Reference Centre for Tick-Transmitted Diseases, Switzerland, 3 ZHAW Spin-off "A&K Strategy GmbH", Neuhaus SG, Switzerland, 4 University of Lausanne, Institut de microbiologie du CHUV, Lausanne, Switzerland, 5 ADMED Microbiologie, La Chaux-de-Fonds, Switzerland

**Background** The tick species *Ixodes ricinus* serves as a vector for numerous human pathogens of bacterial, viral, or protozoic origin. To date, only few studies on pathogen prevalence in ticks parasitizing humans have been performed.

**Methods** Using the smartphone application "Zecke – Tick Prevention" anonymized data on tick bites were collected; some users took the opportunity of sending removed ticks to the Swiss National Reference Centre for Tick-Transmitted Diseases (NRZK / CNRT) for pathogen screening. Using quantitative (RT-) PCR as well as PCR with melting curve analysis or sanger sequencing ticks were screened for the presence of various pathogens.

**Results** In a preliminary study 2017, 84 app users provided ticks for pathogen screening. Overall carrier rates of 0 % for tick-borne encephalitis virus, 7.14 % for *Borrelia burgdorferi* sensu lato, 0 % for *Borrelia miyamotoi*, 16.67 % for *Rickettsia* spp., 3.49 % for *Anaplasma phagocytophilum*, 3.57 % for *Babesia* spp., 1.19 % for *Candidatus* Neoehrlichia mikurensis, and 2.4 % for *Parachlamydia* spp. were found. Carriage of multiple pathogens was found in 2.4 % of all analysed ticks. In 2018, the CNRT laboratory received another 571 ticks from app users. The carrier rates of all 655 ticks will be presented and discussed in September 2019 at the ITPD 2019 in Vienna.

**Conclusion** Our data documents the presence of pathogens in ticks parasitizing humans, with carrier rates comparable to those found in questing ticks. Carriage of multiple pathogens was observed, demonstrating the potential risk of acquiring multiple infections as a consequence of a tick bite.

Links Swiss Reference Centre for Tick-Transmitted Diseases (NRZK / CNRT): https://www.labor-spiez.ch/en/die/bio/endiebionrz.htm
App "Tick prevention" https://www.zhaw.ch/en/lsfm/business-services/institute-of-natural-resource-sciences/ticks/
Institute of microbiology, CHUV, Lausanne http://www.chuv.ch/laboratoires/dl-laboratoire-diagnostic-institut-microbiologie.htm
ADMED Microbiology http://admed.ne.ch/f/page/200/

P93 **Risk mapping and classification for identifying local tick prevention needs in Flemish municipalities**

Katrien Tersago 1, Jim Casaer 2, Johan Brouwers 3, Koen Schoeters 4, Tinne Lernout 1

1 Scientific Directorate of Epidemiology and public health, Belgian institute for health, Sciensano, Brussels, Belgium, 2 Research Institute for Nature and Forest (INBO), Brussels, Belgium, 3 Flanders Environment Agency (VMM), Aalst, Belgium, 4 Agency for Care and Health Flanders, Brussels, Belgium

Risk mapping is often used as a tool for guiding prevention of vector-related issues, even more so when sufficient data on vector hazard or exposure are lacking. In this context, knowledge-based and data-driven risk maps were built for estimating *Ixodes ricinus* - related risk in municipalities in the Flanders region, Belgium. Through objective classification we aimed to divide these municipalities in three groups with different needs for local investment in tick prevention measures.

For knowledge-based risk mapping, a spatial Multi Criteria Decision Analysis (MCDA) with linear weighting was carried out, based on aggregated *Ixodes ricinus* hazard (climate, land use, forest fragmentation and hosts) and exposure (density of walking paths, gardens, open forest areas) risk factors per municipality. Four different weighting scenarios were used in the MCDA.

Data-driven risk mapping was based on tick bite notification data of the citizen-science platform for tick bite surveillance in Belgium (TekenNet). Municipality-specific relative risk (RR) was estimated with a Bayesian convolution model in R-INLA, taking into account uncorrelated heterogeneity, a spatial conditional autoregressive component and ecological risk factors. Objective classification of municipalities in three groups was carried out based on tertiles and exceedance probabilities of RR>1.

The different mapping approaches estimated a very similar spatial risk pattern in the Flanders region. Among the different MCDA scenarios, correlation coefficients varied between 0.93 and 0.99. The MCDA risk estimates were significantly associated with those based on Bayesian disease mapping of tick bite notifications ($p<0.0001$). Hence, in the absence of tick hazard and exposure data, knowledge-based risk mapping can be used for initial prioritization of tick prevention areas. For each of the 3 risk classes, potential tick prevention measures were identified. A study for the evaluation of the practical feasibility of these local prevention measures is planned.

P94 **Annual serological testing for Lyme in a population with high occupational tick bite exposure**

Marlies van Lent, Anke Smits, Kim van den Berg, Anja Garritsen

Innatoss Laboratories, Oss, Netherlands

**Background** Outdoor workers have an increased risk of tick bite exposure and thereby *Borrelia* spp. infections that can lead to Lyme disease. If left untreated, late stage symptoms can develop including neurologic and joint abnormalities. Disseminated Lyme disease leads to high costs for healthcare systems and for society by loss of productivity. Systematic serological testing of outdoor workers enables early detection of *Borrelia* infections in high-risk groups.

**Methods** In the annual screening program, outdoor workers of forestry and water management organizations and green keepers in The Netherlands are tested for *Borrelia* spp antibodies after the tick season. Individual baseline measurements are obtained prior to the tick season, to which subsequent tests are compared to distinguish between old and recent infections. Testing consists of IgG, IgM, and C6 ELISAs, with immunoblot follow-up for positive and dubious samples. Subjects receive a questionnaire on previous Lyme diagnosis, number of tick bites, and antibiotics use.

**Results** Currently 3,000 subjects are enrolled in the screening program, of which 1,358 were at least tested twice. From October 2018 to March 2019, follow-up testing was done for these subjects, who had baseline measurements before the 2018 tick season. In total, 312 subjects (23%) were seropositive (IgG, IgM and/or C6 ELISA). Of these, 29 subjects (2%) had a recent (re-)infection, indicated by increased antibody titers compared to their previous measurement. Subjects had not been aware of infection themselves. Infections were detected in every region in The Netherlands.

**Discussion and conclusion** Among outdoor workers, 1 in 50 subjects contracts a *Borrelia* infection per tick season. Annual serological testing can identify recent infections, enabling early treatment and limiting the risk of late stage Lyme disease symptoms. Moreover, systematic assessment of *Borrelia* seroprevalence provides epidemiological data for different regions and a tool to evaluate the effect of tick bite prevention measures.

P95 **Abiotic and biotic drivers of tick distribution, abundance, and seasonality in urban environments**

Silvia-Diana Borșan*, Andra Toma-Naic, Attila David Sándor, Vasile Cozma, Andrei Daniel Mihalca

Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca

Ticks are increasingly acknowledged as significant vectors for a wide array of pathogens in urban and peri-urban environments, with reports of abundant tick populations in recreational areas. In Romania, information regarding the risk posed by ticks in such areas is absent. In order to assess the risk of human-tick encounter in urban and peri-urban areas, questing ticks were collected by flagging in four types of habitats in Cluj-Napoca, Romania: parks, cemeteries, gardens, and forests. All the areas are open to the public and used for recreational activities. Samplings were performed during the spring, summer and autumn of 2018. Micromammals, hedgehogs and birds were also sampled from the same areas using standard methods (i.e. snap-traps, spot-light, mist nests etc.). Approximately 37,000 m² were covered in total. Overall, 3391 ticks were collected and identified to species level using morphological keys. Only two species of ticks were found: *Ixodes ricinus* (96.8%) and *Haemaphysalis punctata* (3.2%). *Ixodes ricinus* was collected in all locations while *Hae. punctata* was present in all but one location. Ticks were present in all vegetation types, but a positive correlation was established between the abundance of *I. ricinus* and grassy habitats (Pearson, $r=0.681$, $p<0.05$). The presence of *I. ricinus* was also correlated with the presence of hedgehogs (Kolmogorov-Smirnoff, $z= 0.863$, $p<0.05$) and the density of rodents (Pearson, $r=0.815$, $p<0.05$). Since *I. ricinus* is the most common tick feeding on humans in this area and it is the primary vector of important tick-borne diseases in Europe (i.e. *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, tick-borne encephalitis virus), we consider of utmost importance having an overall understanding of the variables of an urbanized environment that shape the intricate tick-host-pathogen relationships in order to develop optimal public health strategies for the prevention and control of vector-borne diseases.

P96 **Ixodes Ricinus, Borrelia afzelii and massive analysis of cDNA ends (MACE), the perfect recipe for an antidote?**

Jos J.A. Trentelman1, Ondřej Hajdušek*2, Nicolas Krezdorn*3, Katsuhisa Takumi4, Radek Šíma2, Björn Rotter3, Hein Sprong4, Veronika Urbanová 2, Peter Winter3, Joppe W.R. Hovius1

Amsterdam UMC, University of Amsterdam, Center for Experimental and Molecular Medicine, Amsterdam, The Netherlands1, Biology Centre of the Academy of Sciences of the Czech Republic (BC ASCR), České Budějovice, Czech Republic2; GenXPro GmbH, Frankfurt Innovation Center Biotechnology, Frankfurt am Main, Germany3; Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands4, * Authors contributed equally

*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 (TSG).

In the current study we measured expression of tick salivary gland proteins (TSGPs) during tick feeding of unfed, 24h fed and fully fed *B. afzelii*-(un)infected *I. ricinus* nymphs by Massive analysis of cDNA ends (MACE) and RNAseq. MACE reads were mapped against contigs obtained by RNAseq, 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 170 transcripts in uninfected TSGs, 291 transcripts at 24 hours after onset of feeding and 465 transcripts in fully fed TSGs.

A total of 20 TSGPs upregulated upon *Borrelia* infection in 24h fed TSG were selected as possible vaccine candidates and their expression profiles was measured in 10 genetically distinct tick pools to assess biological variation. Despite considerable biological variation, 3 TSGPs proved to be significantly and robustly upregulated upon infection in 24h fed TSG across different tick pools. Although these genes are highly upregulated upon *Borrelia* infection, preliminary vaccination with these antigens did not reduce *Borrelia* infection in mice. The roles of these tick salivary proteins in the transmission of *B. afzelii* from the tick to the mammalian host remains to be elucidated.

P97 **Glycerophosphoryl diester phosphodiesterase does not support indirect diagnosis of Borrelia miyamotoi infection**

Michael Reiter[1], Theresa Stelzer[1], Anna M. Schötta[1], Michael Leschnik[2], Anna Harsch[3], Edda Reiß[3], Richard E. Kneusel[3], Hannes Stockinger[1] and Gerold Stanek[1]

[1]Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna
[2]University Clinic for Small Animals, University of Veterinary Medicine, Vienna
[3]DIARECT AG, Freiburg, Germany

The spirochete, *Borrelia miyamotoi* is the causative agent of hard tick relapsing fever borreliosis (HTRFB). *B. miyamotoi* is vectored by the same ixodid tick species as Lyme borreliae (Lb); thus, people exposed to ticks are not only at risk for Lyme borreliosis but also for HTRFB. Several studies have reported HTRFB a flu-like illness with a wide variety of unspecific symptoms. Diagnosis of the disease is challenging and is currently mainly based on serology and/or detection of specific DNA by polymerase chain reaction.

In this study we aimed to determine the seroprevalence of *B. miyamotoi* in Germany and Austria in populations with different exposure to ticks.

Recombinantly expressed *B. miyamotoi g*lycerophosphoryl diester phosphodiesterase (GlpQ) protein was used for detection of antibodies by immunoblotting. We tested populations with frequent tick exposure, as well as and patients with confirmed Lyme neuroborreliosis (LNB). Persons negative for Lb antibodies and healthy blood donors served as controls.

Anti-GlpQ antibodies were detected in the hunter sera (9.4%), in sera of persons with high anti-Lb IgG (23%), and in serum of LNB patients (9.1%). However, we also detected significant amounts of anti-GlpQ antibodies in the control groups: In 14% of persons negative for Lb antibodies we found anti-GlpQ antibodies, whereas 35.7% of healthy blood donors from Germany and 28.6% from Austria were positive.

The high background of anti-GlpQ antibodies in the control groups suggests a high level of cross-reactivity and disproves GlpQ as a specific marker for indirect detection of *B. miyamotoi* infection.

P98 **Prevalence and evolution of Borrelia bavariensis and B. garinii in Eurasia**

Noémie S. Becker, Robert E Rollins, Kozue Sato, Ai Takano, Sergey Kovalev, Hiroki Kawabata, Gabriele Margos and Volker Fingerle

Division of Evolutionary Biology, Faculty of Biology, LMU Munich, Germany, Department of Bacteriology-I, National Institute of Infectious Diseases, Tokyo, Japan, Department of Veterinary Medicine, Joint Faculty of Veterinary Medicine, Yamaguchi University, Japan, Laboratory of Molecular Genetics, Department of Biology, Ural Federal University, Yekaterinburg, Russia. National Reference Centre for Borrelia at the Bavarian Health and Food Safety Authority, Oberschleissheim, Germany

The species complex *Borrelia burgdorferi* s.l. comprises more than 20 described genospecies that differ by their geographic distribution, their tick vector species and their vertebrate reservoir hosts. *B. bavariensis* and *B. garinii* are both present in Eurasia and are genetically closely related species but they use different hosts (rodents and birds respectively) and are found in several tick species depending on their geographic locations. We have sampled questing ticks from 3 populations in Japan, Russia and Germany and have isolated up to 20 strains of *B. bavariensis* and *B. garinii* in each country. We analyzed the prevalence of the two species in each country and show that the prevalences of the two species differ and that *B. garinii* is dominant in Germany. Indeed most *B. bavariensis* strains isolated in Europe to date come from human patients and not from questing ticks. Next, we used whole genome sequencing to reconstruct the plasmid content of each strain. We show that the number of plasmids differs between the two species and between populations with *B. garinii* strains from Asia having more plasmids compared to European strains and to all *B. bavariensis* strains. We also identify plasmids that are specific to each species and thus are good candidates for playing a role in the adaptation to the avian and rodent hosts. Finally, a phylogeny reconstructed using the main chromosome sequences shows that Russian and Japanese isolates of *B. bavariensis* do not constitute isolated populations and that the European population has very low genetic diversity. In conclusion, the study of these two *Borrelia* sister species using strains isolated from randomly sampled questing ticks is a promising framework for understanding the evolution of host and vector adaptation in *Borrelia*.

P99 **Sero-survey for scrub typhus and murine typhus among forestry workers in South Korea**

Sunjin Lee, Kwan Lee, Ji-Hyuk Park, Jeong-Hoon Chun, Kyu Jam Hwang and Seon Do Hwang*

Division of Bacterial Diseases, Center for Laboratory control of Infectious Diseases, Korea Centers for Disease Control and Prevention, Cheongju, Chungcheongbuk-do 28159, Korea / Department of Preventive Medicine, Dongguk University College of Medicine, Gyeongju, Gyeongsangbuk-do 38066, Korea

Scrub typhus is an acute febrile disease characterized by fever, rash, eschar, pneumonitis, and meningitis. It is caused by infection with *Orientia tsutsugamushi*, transmitted by trombiculid mites and occurs frequently in the autumn in Korea. The incidence of the disease has increased gradually to 6,682 cases reported in Korea in 2018. *Rickettsia typhi*, a typhus group *rickettsia*, is the etiologic agent of murine typhus. Although murine typhus is characterized by a low mortality rate (1% of reported cases), in severe cases, murine typhus can result in meningoencephalitis, disseminated vascular lesions. Without specific treatment, 99% of those infected will clear the disease within weeks. In Korea, 16 murine typhus cases were reported in 2018. This serologic study was conducted to obtain basic data on scrub typhus and murine typhus for high-risk populations. This study included forestry workers, considered to be a high-risk population for scrub typhus and murine typhus. For three years, from 2015 to 2018, a total 2,182 forestry workers' sera were collected and antibodies specific to *O. tsutsugamushi* and *R. typhi* were titrated using indirect immunofluorescence antibody assay (IFA). Seroreactivity of scrub typhus were 11.9% (85/715), 10.0% (76/763), 30.6% (216/704) respectively for three years, and that of murine typhus were 26.6% (190/715), 8.9% (68/763), 21.3% (150/704) respectively. This is the serological study of scrub typhus and murine typhus among forestry workers, considered a high-risk population for vector-borne diseases. These result of the seroprevalence shows the high exposure to *R. typhi* and *O. tsutsugamushi* amongst forestry workers. Further analyses of this population for other vector-borne diseases are also needed to identify the risk-status and to improve the control and prevention of these diseases in high-risk populations. This study was supported by the Korean Centers for disease Control and Prevention (4800-4861-304-260-01, 4800-4838-303-210-13).

# Sponsors

**We are grateful to the following companies for their kind support of ITPD 2019**

## Gold Sponsor


# Exhibitors

Euroimmun, Lübeck, Germany

Mikrogen/Neuried, Germany

Quidel, Kornwestheim, Germany

R-Biopharm, Darmstadt, Germany

Reagena, Toivala, Finland

TestLine, Brno, Czech Republic

Viramed Biotech AG, Planegg, Germany

Virotech Diagnostics, Rüsselsheim, Germany

(State at printing)

Printed in Austria by: ROBIDRUCK, 1200 Vienna – www.robidruck.co.at