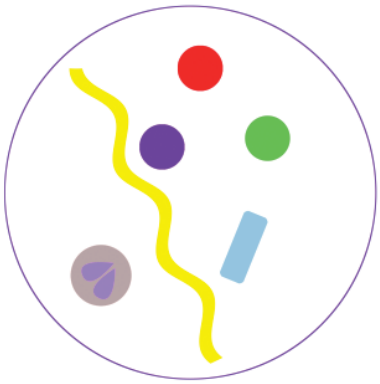

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BOOK OF ABSTRACTS

ÖGHMP
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ABSTRACTS ORAL PRESENTATIONS

Proteomic approaches to identify Ixodes species and its associated pathogens

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Ticks are the most important vectors in human and veterinary medicine. For epidemiological studies, identification of ticks and associated pathogens is generally performed by microscopic observation and/or molecular biology. Nevertheless, these approaches are tedious for tick species determination and not satisfactory for pathogen identification. Indeed, the detection of pathogen DNA does not mean that microorganisms are alive or potentially infectious for vertebrate hosts.

To circumvent these limitations, two proteomic strategies, based on peptide profiling and large-scale protein characterization, were developed for dual identification of tick species and *Borrelia*-infectious status in *Ixodes* specimens.

Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI – TOF MS) was successfully applied for *Ixodes* species identification. The reproducibility and specificity of leg MS spectra from each *Ixodes* species confirmed the accuracy of this simple proteomic approach for tick identification. Unfortunately, MALDI-TOF MS did not succeed in distinguishing MS spectra of pathogen-free *Ixodes ricinus* specimens from conspecific species infected by the Lyme disease agent, *Borrelia burgdorferi* sensu lato. At distance of blood feeding, the gut confinement of *Borrelia* and their low abundance could likely explain the difficulty to detect this pathogenic agent by MALDI-TOF MS.

Then, a large scale proteomic approach was investigated on *Ixodes* ticks collected in Lyme borreliosis endemic region of France for determination of bacteria infectious status. Proteins were prefractionated by gel electrophoresis and identified by LC-MS/MS. Bioinformatic searches were performed using databases including several pathogens that could be transmitted by *Ixodes* ticks. This strategy allowed the identification of *Borrelia* proteins, as well as *Anaplasma*, *Rickettsia* and *Babesia* proteins.

These two proteomic strategies will be useful for precise epidemiological studies on tick-borne diseases allowing the establishment of infectious risk map or vector control measures.

Pandora's flying box - Borrelia burgdorferi sensu lato prevalence in Ixodes species from birds throughout Europe

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Birds are important hosts for ticks and may act as reservoirs for several zoonotic pathogens. Because of their high mobility, especially of the long distance migratory species, they can act as dispersers for ticks and pathogens, ultimately affecting their distribution and phylogeography.

Ixodes ticks (n = 2258) were collected from passerine birds either captured in mist nets or breeding in nest boxes from 2006 to 2016 from 10 countries in Europe, covering an area from 8º23'W to 24º57'E and from 62º14'N to 40º35'N. Most of the ticks were identified morphologically as *Ixodes ricinus* (n = 1803), followed by *I. arboricola* (n = 217) and *I. frontalis* (n = 160). *I. ventalloi* (n = 4) and *I. apronophorus* (n = 1) were also found. DNA was extracted from a representative sample of these ticks (n = 667) and infection by *Borrelia burgdorferi* s.l. was analysed by a nested PCR targeting the *flaB* gene. *Borrelia* prevalence was 36.6% (244/667), and between countries it varied from 20.8% (Portugal) and 51% (Sweden). *Turdus pilaris* was the bird species that carried ticks with the highest *Borrelia* prevalence (92.8%), followed by *Turdus merula* (59.5%).

B. garinii was the most prevalent genospecies (62.3%; 119/191), followed by *B. valaisiana* (23.0%; 44/191), *B. afzelii* (8.4%; 16/191), *B. turdi* (5.2%; 10/191) and *B. lusitaniae* 0.52% (1/191). *B. turdi* was detected in three species of ticks (*I. frontalis*, *I. ricinus* and *I. ventalloi*) infesting *Turdus* sp. and *Parus major*. *B. garinii* genotypes detected from birds of different European regions and with different migratory movement ranges were characterised by multilocus sequence typing and compared to assess variability within this genospecies and potential phylogeographic pattern.

Ixodes ricinus winter survival and spring emergence in Norway

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Ixodes ricinus typically quest in Norway from April to October, however host-seeking activity also occur during winter months under mild weather conditions. It has been suggested that milder winters may lead to an extended seasonal activity for *I. ricinus.* As Norway is a part of the northern border for *I. ricinus*' geographical range, knowledge from this region will be of great importance when studying the possible effect of climate and climatic changes on tick activity and distribution. The present study started in 2014, aiming at studying the winter survival, spring emergence and host-seeking activity of *I. ricinus* at three forest sites in southern and southeastern Norway. A semi-natural system for the observation of tick host-seeking activity was used. Field-collected nymphal ticks were fed a full blood meal from sheep before they were released fully engorged into field plots in June each year. The tick plots were monitored twice a week throughout the study period. Data loggers were placed at ground level inside the plots and at approximately 30 centimeters height next to the plots to measure temperature and humidity at an hourly basis. These environmental data, as well as data on temperature and precipitation from nearby meteorological stations, were used to assess the between-year and between-site variation in tick winter survival and onset of questing in spring. The moulting was completed and activity commenced after 33-45 weeks (ultimo January – medio April) after introducing the engorged nymphs in the plots. The winter survival varied greatly between sites and years, and was highly dependent on local winter temperature.

Competition between strains of Borrelia afzelii in the rodent host and the tick vector

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Many populations of vector-borne pathogens consist of multiple genetically distinct strains. These genetically diverse pathogen populations often establish multiple strain infections inside their vertebrate host as well as their arthropod vector. Multiple strain infections can result in competitive interactions between strains that reduce their abundance and transmission success. These competitive interactions may play an important role in shaping the strain structure of vector-borne pathogen populations in the field. *Borrelia afzelii* is a tick-borne spirochete bacterium that is one of the most common causes of Lyme borreliosis in Europe. In nature, multiple strain infections of *B. afzelii* are common in both the tick vector *Ixodes ricinus* and the rodent reservoir host. Here we used two genetically distinct strains of *B. afzelii*, to investigate the interactions of mixed strain infections inside the rodent reservoir host and the tick vector. Mice were infected via tick bite with either one strain or co-infected with both strains. The infected mice were infested with larval ticks from our pathogen-free *I. ricinus* colony, and the engorged larval ticks were allowed to moult into nymphs. These nymphs were tested with strain-specific qPCR assays to determine the host-to-tick transmission success of each strain and the spirochete load of each strain inside the nymphs. We found evidence of competitive interactions with respect to host-to-tick transmission and the spirochete load inside the nymphal tick. Competition reduced host-to-tick transmission of one strain by almost 50% whereas the other strain was not affected. For both strains, we found that their spirochete load was reduced by 50% in the presence of the co-infecting strain. The present study therefore demonstrates that *Borrelia* strains compete for limiting resources inside the tick vector. Future studies should investigate whether this competition influences the tick-to-host transmission success of each strain.

Ixodes pavlovskyi: known tick species, unknown vector

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The *Ixodes pavlovskyi* tick species, a member of the *I.persulcatus/I.ricinus* group, was discovered in the middle of the 20th century in the Far East. This tick species has a discontinuous distribution area, including the Far Eastern and Western Siberian regions. Last years, an increased abundance of *I.pavlovskyi* ticks has been recorded more northward in Western Siberia, and *I.pavlovskyi* ticks have become predominant in suburban areas of Novosibirsk and Tomsk, large Siberian cities, where they frequently attack people. Recently, the existence of natural hybrids of *I.pavlovskyi/I.persulcatus* in sympatric areas was demonstrated. Limited data have been reported on the detection of infectious agents in *I.pavlovskyi* ticks and its hybrids. The aim of this study was to investigate the prevalence and genetic variability of a wide range of infectious agents in *I.pavlovskyi* ticks and *I.pavlovskyi/I.persulcatus* hybrids compared to *I.persulcatus.*

A total of 2337 *Ixodes* ticks were collected in *I.pavlovskyi* traditional and recently invaded habitats, the Altai Mountains and Novosibirsk Province, respectively, where *I.pavlovskyi* and *I.persulcatus* ticks exist in sympatry. Tick species and *I.pavlovskyi/I.persulcatus* hybrids were confirmed by the nuclear and mitochondrial loci. Tick-borne encephalitis and Kemerovo viruses, *Borrelia* spp., *Rickettsia* spp*.,* Anaplasmataceae bacteria, and *Babesia* spp. have been detected by RT-PCR or PCR and genetic diversity of the infectious agents was determined by sequencing.

The prevalence and genotypes of many of the identified agents did not significantly differ between *I.pavlovskyi*, *I.persulcatus*, and *I.pavlovskyi/I.persulcatus* hybrids. However, *I.pavlovskyi* ticks were significantly more often infected by *B.garinii* and less often by *B.bavariensis*, *B.afzelii*, "*Ca. R.tarasevichiae*", and *E.muris* than *I.persulcatus* ticks in both studied regions. The prevalence of the agents in *I.pavlovskyi/I.persulcatus* hybrids was intermediate. In addition, new genetic variants of *B.burgdorferi* s.l. and *Rickettsia* spp. as well as tick-borne encephalitis and Kemerovo viruses were found in *I.pavlovskyi, I.persulcatus* ticks and their hybrids.

Molecular detection of tick-borne pathogens in humans with a tick bite or erythema migrans, in the Netherlands

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Knowledge on the incidence and clinical presentation of tick-borne diseases other than Lyme borreliosis and tick-borne encephalitis is minimal. Using molecular detection techniques, we estimated the frequency of other tick-borne infections than Lyme spirochetes in patients with a tick bite or erythema migrans.

Ticks, blood samples and questionnaires were collected from patients that visited their general practitioner with a tick bite or erythema migrans in 2007 and 2008 in the Netherlands. The presence of several tick-borne pathogens in 314 ticks and 626 blood samples of these participants were analysed, using PCR-based methods. Using multivariate logistic regression, associations were explored between DNA of pathogens detected in blood and self-reported symptoms at enrolment and during a three-month follow-up period.

47% of the ticks carried DNA of one or more tick-borne pathogens, such as *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Candidatus* Neoehrlichia mikurensis, *Rickettsia helvetica*, *Rickettsia monacensis*, *Borrelia miyamotoi* and several *Babesia* species. 33% of 92 *Borrelia burgdorferi* s.l. positive ticks carried another pathogen from a different genus. In blood of sixteen out of 626 participants, DNA was detected from *Candidatus* Neoehrlichia mikurensis (n=7), *Anaplasma phagocytophilum* (n=5), *Babesia divergens* (n=3), *Borrelia miyamotoi* (n=1) and *Borrelia burgdorferi* s.l. (n=1). None of these sixteen individuals reported any overt symptoms that would indicate a corresponding illness during the three-month follow-up period. No associations were found between detection of pathogen DNA in blood and self-reported symptoms, nor with pathogen DNA in the corresponding ticks, reported tick attachment duration, tick engorgement, or antibiotic treatment at enrolment.

With molecular detection techniques, the probability of infection with a tick-borne pathogen other than Lyme spirochetes is roughly 2.4% after a tick bite, and 2.7% co-infections among patients with erythema migrans. How often these infections cause disease symptoms or to what extend co-infections affect the course of Lyme borreliosis needs further investigation.

Estonia - an endemic region of major tick-borne infections: situation over a decade

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Over the last decades Estonia has one of the highest morbidity rates of both tick-borne encephalitis (TBE) and Lyme borreliosis (LB), with incidence rates up to 19,8 and 171,8 cases per 100 000 population, respectively. Borrelia miyamotoi disease is also of an increasing public health interest but no human cases reported up to date.

Estonia contributes a unique region for the investigation of ticks and tick-borne pathogens (TBPs) as *Ixodes ricinus* and *I. persulcatus* co-distribute in the Eastern and Southern parts of the country. Rodent specialist species *I. trianguliceps* which plays role in the circulation and maintenance of TBPs, has been also found in Estonia.

In the years 2006 - 2014 ticks collected for several projects from vegetation, small rodents and migratory birds had been analyzed for TBEV, *Borrelia burgdorferi* s.l. and *B. miyamotoi* presence. Also, a novel single tube multiplex PCR assay for discrimination of *I. persulcatus*, *I. ricinus and I. trianguliceps* has been developed to overcome morphological identification misinterpretations. It has been shown that site-specific prevalence rates of TBPs in ticks from vegetation were up to 4.4% for TBEV, up to 40.2% for Lyme *Borrelia* and up to 2.9% for *B. miyamotoi*. Besides, for all these TBPs prevalence rates are mostly significantly higher as for *I. persulcatus*, compared to *I. ricinus*, as well as for sympatric areas, where this tick species co-distributes with *I. ricinus*, compared to to *I. ricinus* allopatric area. There were TBEV-Eu,TBEV-Sib and TBEV-FE subtypes, five genospecies of *B. burgdorferi* s.l. and relapsing fever group *B. miyamotoi* found. TBEV-Sib was detected not only in its associated vector, *I. persulcatus*, but also in *I. ricinus.* Phylogenetic analysis of TBEV partial E and NS3 genes revealed that Estonian TBEV-Sib strains from both *I. persulcatus* and *I. ricinus* cluster together within Baltic sublineage of TBEV-Sib.

Molecular eco-epidemiology of borreliosis in Slovakia

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The aim of the study was to identify the prevalence and genetic variability of *Borrelia burgdorferi* s.l. and occurrence of *B. miyamotoi* in questing *I. ricinus* ticks in various habitats of Slovakia. Moreover, we have tested the *Borrelia* prevalence and its heterogeneity in ticks that were detached from patients. In total 1355 out of 7739 (18%) questing *I. ricinus* ticks were infected with *B. burgdorferi* s.l. which corresponds to the mean European prevalence. *B. afzelii*, *B. garinii*, and *B. valaisiana* were detected at each studied site as the most prevalent with the few exceptions. In sub-mountain area of central Slovakia, *B. lusitaniae* predominated. *B. burgdorferi* s.s.was found in urban parks from both western and eastern Slovakia. At these sites, *B. bavariensis* and *B. spielmanii* were detected. *Borrelia*-positive ticks in urban habitats harbored mostly *B. garinii* and *B. valaisiana* assigning blackbirds population an essential role for circulation of borrelia in towns. The lowest prevalence of *Borrelia* was found at urban area with very low density of rodents where deer supplemented the blood meal for the *I. ricinus* larvae. *B. myiamotoi* was detected in 23 out of 2969 (0.8%) tested questing ticks from various sites in Slovakia. 18.1% (41/226) ticks removed from patients were positive for *B. burgdorferi* s.l. in contrast *B. myiamotoi* was found only in 2 ticks. Higher prevalence of *B. burgdorferi* s.l. was found in adult females (32.7%) as in nymphs (16.8%). *B. afzelii* predominated, followed by *B. garinii*, *B. valaisiana*, *B. lusitaniae* and *B. spielmanii*. MLST analysis revealed presence of already known sequence types of *B. afzelii* and *B. garinii*. *B. lusitaniae* were closely related to Serbian isolates deposited in MLST database. Based on the concatenated sequence of seven houskeeping genes these strains represented an unique genotype not previously detected in Europe.

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Estimating the risk associated with Ixodes ricinus parasitism: towards the development of serological markers for the tick bite

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Ixodes ricinus is the most important ectoparasite in Europe, responsible for the transmission of several pathogens. The characteristics of the tick bite (e.g. absence of immediate symptoms) imply the possibility that part of the infections transmitted by ticks remain occult. It is thus difficult to estimate the actual impact of ticks on the health of human and animal populations.

A tool capable of determining whether humans/animals have been bitten by *I. ricinus* would allow to: determine the actual 'pressure' of the tick bite; estimate the risk associated with tick parasitism; contribute to the diagnosis of diseases such as the Lyme disease, where anamnestic information is frequently lacking.

With the goal of developing markers for the 'diagnosis' of the *I. ricinus* bite, we first focused on *Midichloria mitochondrii*, a bacterium present in all the infecting stages of *I. ricinus,* reaching 100% prevalence in adult females, where it is also observed in the salivary glands. Humans and animals exposed to *I. ricinus* present an antibody response against a recombinant flagellar protein (rFLID). rFliD might thus represent an antigen for the serological evaluation of the exposure to the tick.

Seroprevalence for *Midichloria* rFliD in humans exposed to *I. ricinus* ranges from 30 to 60%. Thus, rFliD appears promising, but incapable of determining the exposure to *I. ricinus* in approximately 50% of the patients. We initiated a seroproteomic work on *I. ricinus* and *Midichloria* proteins, with the goal of detecting further antigens. Using sera from patients parasitized, collected at the tick removal and after 40-60 days, we characterized additional proteins that determined seroconversion. Work is in progress to develop peptides from the discovered antigens, in order to set-up an ELISA-assay for a first application and validation of this serological tool, on a wide panel of subjects exposed at a different risk of tick parasitism.

Tick saliva and its role in pathogen transmission

[Key note lecture]

Pat Nuttall

University of Oxford

Tick saliva contains 100s of different proteins, peptides, and non-peptidic molecules. Particular protein folds – notably Kunitz and lipocalin – are enriched in tick saliva. The *Ixodes scapularis* tick genome reveals an expanded repertoire of proteins containing a Kunitz domain (74 genes, 0.4% of the predicted proteome), implicated in protease inhibition and channel-blocking activity, with roles in inhibiting coagulation, angiogenesis and vasodilation. Lipocalins are small, secreted proteins that carry even smaller ligands or substrates. Although the tick genome is the richest source of lipocalin genes to date, most tick saliva lipocalins have unknown function. Nevertheless, all bioactive molecules secreted in tick saliva are designed to overcome their hosts' efforts to prevent ticks feeding on them. Besides the physical barrier of the skin, the bloodthirsty tick must overcome chemical and cellular host response mechanisms of haemostasis, inflammation, and immunity. These 'smart pharmacologists' (as they have aptly been called) secrete, into the skin-feeding site, anaesthetics, anti-coagulants, and molecules that control the inflammatory/immune host responses. Synthesis of these molecules in the salivary glands is tuned to the host response, with different molecules secreted at different times of the relatively long (up to 10 days or more) period of attachment and feeding. The effects on the host of these bioactive saliva molecules not only help the tick to imbibe its enormous blood meal but also facilitate transmission of tick-borne pathogens. Probably all tick-borne pathogens have evolved to exploit the bioactivity of their tick vector's saliva. They include the most important tick-borne pathogens affecting humans such as Crimean Congo haemorrhagic fever virus, tick-borne encephalitis virus, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sl, and *Franciscella tularensis*. Unravelling the interactions between tick saliva, pathogen, and host is providing new insights into the challenge of controlling tick-borne infections and associated diseases.

The sticky aspect of ticks – the tick cement plug for bionic bioadhesive research

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In medicine ticks are mainly studied for their role as vectors of pathogens causing numerous tick-borne diseases. However, ticks have also developed substances that are interesting for a potential use in clinics such as anticoagulants, pain inhibitors or immunomodulators. And, there is a sticky substance called cement that is deposited around the mouthparts working like a plug to stabilize the ticks on the host. Due to its adhesive properties under wet conditions and to tissue as such, the material gained interest for biomedical research on biological adhesives. In future the sticky substance could serve as template to produce an alternative tissue glue. There is a need for clinical glues, since those which are currently used in clinics either contain toxic substances causing tissue necrosis and local inflammation, or they are well biocompatible but have a low bonding force. This is the reason why bioadhesive research is a growing field in biomedical basic and translational science. Organisms of many genera produce glues to adhere to surfaces, catch pray or defend against predators. For some organisms such as the *Mytilus* mussel, it was already possible to define and rebuild the adhesive molecule of their byssal threads (3,4-Dihydroxyphenylalanine (DOPA)). However, despite the biocompatibility of this adhesive substance and its stronger bonding force than fibrin-glue, DOPA-based adhesives do not cover all requirements of tissue glues. The identification of other biological adhesives is therefore the aim of several research projects including the tick cement study. After characterisation of the substance and identification of the adhesive component, it may be produced either synthetically or by recombinant expression. We want to provide an overview on research activities in the field of bioadhesives for medical application with special focus on tick cement.

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Active water vapour uptake in unfed and engorged ticks at cold temperatures and ecological implications

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Active water vapour uptake (WVU) is the key capability of unfed and some engorged tick species to maintain water balance in a mostly dehydrating atmosphere, i.e. at ≤99% RH. It is well known that ticks use this specialised way of obtaining water above 80–85% RH down to approximately 5–10°C. Winter temperatures in the temperate climate zone, however, are usually lower, so the question arises how ticks maintain water balance in extended periods of less than 5°C. This was investigated by exposing unfed and engorged ticks of different species and life stages to high and low RHs at low temperatures and weighing them intermittently on a microbalance. Usually, active WVU results in a measurable body mass gain in ticks. Unfed *Ixodes ricinus* adults were capable of achieving considerable body mass gains down to –1°C (at ~94% RH), the lowest temperature investigated. Engorged, diapausing larvae and nymphs of *I. ricinus* gained atmospheric water vapour at temperatures as low as approximately 0.5°C and 0°C, respectively. Unfed and engorged, diapausing nymphs of *I. scapularis* were capable of WVU down to approximately 2°C and engorged, diapausing nymphs of *Haemaphysalis punctata* at approximately 0°C. Engorged, diapausing *Argas reflexus* N1 nymphs and an unfed female gained water vapour at approximately 1°C and 2°C, respectively. These results indicate that WVU can be a very effective mechanism of ticks for maintaining water balance even during the cold season and probably is a key factor for the overwintering of many tick species.

Red(y) to be seen: The transformation and visualization of a fully infectious fluorescent European species of Lyme disease Borrelia, Borrelia afzelii

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The Lyme disease spirochete *Borrelia afzelii* is the most prominent pathogenic *Borrelia* species in Europe. The genes encoded within its highly segmented genome made up of linear and circular plasmids apart from the chromosome, help the spirochete disseminate and maintain itself in a zoonotic cycle that includes the primary tick vector in Europe, *Ixodes ricinu*s and various mammalian reservoir hosts. The absence until now for the application of adapted genetic techniques for any European *Borrelia* species has meant a limited understanding of the role of various genetic factors in this particular spirochete species zoonotic life cycle. The publication in 2011 of the annotated genomes of two isolates of *B. afzelii* made this objective more feasible for various reasons including the determination of plasmid profiles during genetic manipulations. We have been able to modify a whole host of genetic tools and adapt them for various European *Borrelia* species. We can now constantly grow the *B. afzelii* CB43 strain on solid media and thus have been able to obtain individual clones whose plasmid profiles are known as well as their infectivity in mice and ticks. Our success in being able to transform the strain CB43 alongwith other European *Borrelia* species using shuttle vectors and obtaining infectious transformants has allowed us to push the boundaries of studies with European species. We have had success in obtaining fluroscent dsRED expressing CB43 which we show to be fully infectious within the tick-mouse model. The use of fluorescent spirochetes has opened new avenues and with the use of confocal microscopy and CLEM (Correlative Light Electron Microscopy) we will be able to possibly shed some light on some new tick-associated genetic determinants of *B. afzelii* in vivo. We believe that characterization of such genetic determinants responsible for spirochete migration/transmission will lead to a better understanding of ways to actively hinder these routes/processes.

Canine breed predisposition in tick-borne diseases

[Key note lecture]

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Dogs are frequently infested by ticks, thus may be infected by tick-borne pathogens. As specific immune response influences the occurrence of clinical signs, breed associated immune competence may lead to breed predisposition in tick-borne diseases (TBD) in canines. Literature review on canine borreliosis and tick-borne encephalitis as well as current data on canine anaplasmosis and babesiosis represent the basis for this report. Canine borreliosis is a rare clinical disease but a specific borrelia associated Lyme nephropathy in Bernese mountain dogs has been described and possibly a genetic podocytopathy or immunodysregulatory defect triggers abnormal immune complex handling also in other breeds. Several reports on tick-borne encephalitis in dogs refer to two breeds more often: the Siberian Husky and the Rottweiler, although the total reported case number in canine tick-borne encephalitis is low. For canine anaplasmosis (n=293) and babesiosis (n=357) breed data of infected dogs were compared to a breed data set of Viennese dogs (n=30647) from 2016 and canine patients of the Veterinary University Vienna (n=66903) from 2001-2016. Breed data sets of >500 (35 out of 131 breeds) were analyzed. Mixed breed dogs revealed a cumulative incidence of 0.35-0.65 for anaplasmosis and 0.67-1.24 for babesiosis, similar to the overall population (0.44-0.96; 0.53-1.16 respectively). The maximal cumulative incidence was 1.97 – 5.88 for anaplasmosis in Golden Retriever and Hovawart, and 2.53 – 20.51 for babesiosis in Munsterlander and German wire-hair pointer. Existing data on breed related immune system diversity lead to the conclusion that inbreeding may be one cause of breed predisposition for TBD as e.g. hunting usage does not explain all breed related TBD incidence.

The complement binding and inhibitory protein CbiA of Borrelia miyamotoi degrades extracellular matrix components by interacting with plasmin(ogen)

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The relapsing fever spirochete *Borrelia* (*B.*) *miyamotoi* is transmitted by ixodid ticks and causes the hard tick-borne relapsing fever. More recently, we have identified a surface-exposed molecule, CbiA exhibiting complement binding and inhibitory properties, which rendered spirochetes resistant to complement-mediated lysis. To gain deeper insight into the molecular principles of *B.miyamotoi*-host interactions, we examined CbiA as a plasminogen receptor and potential adhesin that enables *B.miyamotoi* to interact with the serine protease plasminogen and to adhere to mouse brain microvascular endothelial cells. Our data clearly show that CbiA was able to bind plasminogen in a dose-dependent fashion. Moreover, lysine residues appear to play a role in the protein-protein interaction, as binding of plasminogen could be inhibited by the lysine analog tranexamic acid as well as by increasing ionic strength. Of biological relevance, plasminogen bound to CbiA can be converted by urokinase plasminogen activator (uPa) to active plasmin which cleaved both, the chromogenic substrate S-2251 and more importantly its physiologic substrate, fibrinogen. Concerning the involvement of specific amino acids in the interaction of plasminogen, lysine residues located at the C-terminus are frequently involved in the binding as reported for various plasminogen-interacting proteins of Lyme disease spirochetes. Since we were unable to sufficiently purify C-terminally truncated CbiA constructs, lysine residues located within the C-terminal domain were substituted to alanine to generate single, double and triple substitution mutants. However, binding of plasminogen to the mutated CbiA proteins was not affected suggesting that lysine residues distant from the C-terminus might be involved in the interaction. In addition, transformed borrelial cells producing CbiA at their surface adhered to mouse brain microvascular endothelial cells to a significant higher amounts compared to the wild-type borrelial strain suggesting that CbiA may play a role in the adherence of *B. miyamotoi* to the vasculature of the human brain.

Novel approaches shed light on transcriptome architecture and gene regulation in Borrelia burgdorferi: a new look at an old picture

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During its natural enzootic lifestyle, *Borrelia burgdorferi* cycles between *Ixodes* spp. ticks and vertebrates. To accomplish this cycle, *B. burgdorferi* must successfully survive and transmit between these two niches. This success relies on dynamically altering the transcriptome for each particular environment. While much progress has been made towards understanding the gene regulatory adaptations required for success, significant questions regarding the organization of key gene regulatory networks (GRN) remain.

RNA-Seq has revolutionized the study of transcriptomics and biology; allowing rapid detection of gene expression and discovery of novel transcripts. We have used this methodology to define the *B. burgdorferi* transcriptional landscape and map essential GRNs.

We analyzed the transcriptome of *B. burgdorferi* to identify 5' transcript ends and transcriptional stop sites on a genome-wide scale. Previously, only a handful of non-coding RNAs (ncRNA) had been discovered. We identified over 350 putative ncRNAs, vastly expanding the possible mechanisms of gene regulation. These findings serve as a foundational resource for the field of *B. burgdorferi* gene regulation.

We are performing differential transcriptomics between mutants of transcription factors (TFs) important for virulence. We analyzed mutants in RpoN, RpoS, CsrA, and BadR; all of which are essential for the enzootic cycle. We found that deletion of BadR or CsrA resulted in drastic transcriptomic alterations. In addition, these two TFs convergently regulate nearly 100 transcripts, including numerous ncRNAs. BadR has a role in the transcriptional control of carbohydrate metabolizing genes, while CsrA exerts a strong transcriptional impact on virulence-associated genes. Intriguingly, we identified a number of transcripts that were previously hypothesized to be dependent upon the alternative sigma factors (i.e. *ospC* and *dbpB*), were regulated by CsrA independently of either sigma factor.

Our long-term goal is to identify points of intersection between key TF networks in *B. burgdorferi*. These points of convergence likely represent novel and unique targets for interventions.

Experimental infection of voles from nature with Borrelia miyamotoi

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Only a few *in vivo* models for *Borrelia miyamotoi* infection have been described to date. After laboratory infection with *B. miyamotoi* or other relapsing fever *Borrelia*, spirochetemia lasts until three or in rare cases up to 10 days in immunocompetent mice and persisted in immunocompromised mice until death. Rodents are infected *with B.miyamotoi* in nature; however the characteristics of this infection and the role of certain species as *B. miyamotoi* reservoir hosts are not yet clarified.

We aimed to investigate the bacterial load after experimental infection of voles *(Myodes rutilis)* captured in nature. Eight adult voles were inoculated i.p. with 10^7 *B. miyamotoi* spirochetes, either clinical isolate Izh-4 or Yekat-1. Subsequently, 0.1 ml blood samples were taken 15 times between day 2 to 47 post-infection and subjected to quantitative real-time PCR with a detection limit of 50 *B. miyamotoi* genome copies/ml. No visible signs of malaise were observed in infected animals.

Despite individual differences, several common characteristics of bacterial load were found among infected animals. Four peaks in spirochetemia were observed: 2 to 4 days after infection (concentration about 150000 copies/ml), sometime between days 7 to 15 (about 60000 copies/ml), between days 18 to 36 (about 1200 copies/ml), and finally between days 37 to 43 (about 400 copies/ml). Between peaks, the *B.miyamotoi* load decreased by a thousand times and was undetectable between peaks 3 and 4. At day 47 the bacterial load was below (7 voles) or slightly higher than the detection limit (one vole).

Future studies will determine (1) whether *Borrelia* infection was completely eliminated or whether latent infection can occur and reactivate in certain conditions and (2) whether changes in bacterial load are related to the changes in expression of *B. miyamotoi* antigens.

This study was supported by the grant of Russian Scientific Foundation (project 15-15-00072).

REV-A-lutions: contributions of fibronectin-binding adhesins to distinct aspects of Borrelia burgdorferi pathogenesis

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The tick-borne pathogen *Borrelia burgdorferi* causes Lyme disease, a significant public health concern across the northern hemisphere. *B. burgdorferi* is extraordinary among bacteria in its ability to invade, disseminate, and persist in their hosts. *B. burgdorferi* expresses numerous adhesins that interact with diverse components of the host's extracellular matrix and cell surfaces. *B. burgdorferi* has at least five fibronectin-binding adhesins, including BBK32, and three which we have identified and characterized: BB0347, RevB, and RevA. Despite seemingly redundant properties of Fn-binding adhesins, each of these proteins likely serves distinct or complementary functions in colonization, persistence and immune evasion. To further define the role(s) of RevA during mammalian infection, we created a mutant that is unable to produce RevA. We demonstrated that the mutant is deficient in colonization of the heart, has more severe arthritis, with increased fibrotic collagen deposition, and has increased levels of serum chemokines and cytokines. These data demonstrate that RevAhas distinct effects on dissemination, arthritis severity, and host response. We have now created a mutant that is unable to produce both RevA AND BBK32. We hypothesize that individual Fn-binding adhesins play distinct roles in colonization, dissemination, persistence, and immune modulation. Our ongoing studies will define the contributions of fibronectin-binding adhesins to multiple aspects of *B. burgdorferi* pathogenesis. These studies will illuminate the mechanisms that *B. burgdorferi* employs to establish and maintain a long-term presence in the host. Given the importance of fibronectin-binding proteins to many bacteria, understanding the contribution of these adhesins may provide crucial information about dissemination and persistence of other extracellular pathogens.

In vitro cultivation of Candidatus Neoehrlichia mikurensis from clinical isolates

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Candidatus Neoehrlichia mikurensis is an emerging tick-borne pathogen that causes the infectious disease neoehrlichiosis. Since the bacterium is a strict intracellular pathogen, it does not grow in cell-free media and is consequently missed by routine microbiological diagnostic methods such as blood culture. The aim of this study was to isolate and propagate *Candidatus* Neoehrlichia mikurensis in cell lines. Infected material was inoculated into the promyelocytic leukemia cell line HL60, recommended for membersof the family *Anaplasmataceae*, three different human endothelial cell lines (derived from umbilical vein, aorta and coronary arteries) as well as on to tick cell lines (derived from *Ixodes ricinus* and *Ixodes scapularis)*. Cultivation attempts were made using homogenates of *Neoehrlichia*-infected ticks, hemolymph from infected ticks and blood specimens from neoehrlichiosis patients. Growth in the cell cultures was monitored using a *Neoehrlichia*-specific real-time PCR assay. Multiple culture attempts were unsuccessful. Finally, we succeeded in cultivating *Candidatus* Neoehrlichia mikurensis following inoculation of blood from two different immunosuppressed neoehrlichiosis patients into the tick cell lines IRE/CTVM20 and ISE6. It took 5.5 months for the cell cultures to become detectably infected and, at the time of writing, the bacteria have been growing in culture for 8 months. The specificity of the infection was verified both by PCR and by labelling the infected cells with panbacterial and *Neoehrlichia*-specific fluorescent DNA probes followed by analysis using Image flow cytometry. To conclude, we report the successful *in vitro* cultivation of two clinical isolates of *Candidatus* Neoehrlichia mikurensis in tick cell lines. This advance takes us one step closer to removing “Candidatus" from this bacterial species' name and will hopefully facilitate research within the field of *Neoehrlichia*.

Borreliella - are there benefits from dividing the genus Borrelia?
[Key note lecture]

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The genus *Borrelia* proposed in 1907 by Swellengrebel (1) includes species of the relapsing fever group of spirochetes, reptile-associated spirochetes and the Lyme borreliosis group of spirochetes. A division of the genus *Borrelia* was proposed based on genomic insertions/deletions (indels) and conserved signature proteins of genome sequence data available in GenBank of relapsing fever and the Lyme borreliosis group of spirochetes (2). These authors suggested keeping the name *Borrelia* for relapsing fever spirochetes while a new genus "*Borreliella*" was proposed for species belonging to the Lyme borreliosis group of spirochetes.

As a consequence of this genus division, eight of the 20 genospecies of the *B. burgdorferi* sensu lato complex were renamed in the List of Prokaryotic Names with Standing in Nomenclature (LPSN), an instrument of the International Committee on Systematics of Prokaryotes to record validly published bacterial names. Public databases such as GenBank started to use the new name for genome sequences of LB spirochetes. However, the new species designations are not used for deposited nucleotides. The evidence provided for the genus division of *Borrelia* was not met with agreement in the research and medical community (3).

According to the rules of the International Code of Nomenclature of Bacteria both names, *Borrelia burgdorferi* and *Borreliella burgdorferi*, can be used synonymously. The implications of this and the benefit of the genus division of Borrelia will be discussed.

(1) Swellengrebel (N.H.) 1907. Annales de l'Institut Pasteur (Paris) 21, 562-586.

(2) Adeolu, M. and Gupta, R. S. 2014. Antonie van Leeuwenhoek 105, 1049-1073.

(3) Margos, G.; Marosevic, D.; Cutler, S.; Derdakova, M.; Diuk-Wasser, M.; Emler, S.; Fish, D; Gray, J.; Hunfeld, K. P. and Jaulhac, B., et al. 2017. IJSEM 67(4), 1081-1084

Probabilistic diagnostics of neuroborreliosis without “Possible NB"

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The case definition of central neuroborreliosis (NB) mostly used in Europe, proposed by EFNS, depends on clinical and serologic data. This diagnostic process generates a large group of possible NB cases, resulting in uncertainty. In the Åland Islands the annual infection incidence is >1500/10^5 inhabitants. This epidemiological situation results in a population seroprevalence of 20 – 60% with important impact on supportive laboratory results on diagnostics. In the present cross-sectional study the development and validation of a probability based diagnostic process will be described.

A derivation cohort of 6530 patients during 2010 – 2013, referred for borrelia serology, estimated a priori pre-test probability 0.01. Clinical information, serology data in serum /CSF as well as CSF-CXC13 were retrieved. A binary diagnostic algorithm was constructed. The diagnostic performance was validated in a cohort of 3370 patients during 2013 – 2014.

C6-peptide antibodies were quantitated by C6 Lyme ELISA Kit (Immunetics, USA), IgG-antibodies to recombinant borrelia antigens by ELISA (recomWell, Mikrogen, Germany) and verified by recomBead, (Mikrogen).

IgG specific intrathecal antibody index was determined by recomBead according to Reiber. CSF-CXCL13 was quantitated by ELISA (Quantikine R&D, USA).

In the derivation cohort the pre-test probability of NB was 0.012. Detection of antibodies in serum raised the probability to 0.039. A CSF study irrespective of antibody status was performed in 8.2% and seropositivity raised the probability of NB to 0.14 in this subgroup. NB according to EFNS were 17% and possible NB 40%. The stepwise calculation of post-test p for NB attained >0.9 and possible NB cases were eliminated. The validation and derivation sets performed identically in logistic regression.

Antibiotic treatment and long-term outcomes of patients with disseminated early Lyme borreliosis

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Background Although several guidelines advocate the same treatment approach for multiple erythema migrans (MEM) as for solitary EM, this recommendation is not well substantiated. Information on direct comparison of the course and outcome of MEM and EM is limited.

Methods 14-day treatment with oral doxycycline 100 mg bid was compared on a non-inferiority premise to 14-day intravenous ceftriaxone 2 g od in adult patients with MEM. The causative *Borelia* species, clinical course and outcome during 12-month follow-up period in patients with MEM vs. patients with EM were investigated. Nonspecific symptoms in patients and controls without a history of Lyme borreliosis were evaluated and compared.

Results 543 patients were enrolled in this prospective study. Outcome in 90 MEM patients treated with doxycycline was not inferior to outcome in 90 MEM patients who received ceftriaxone, nor was there any significant difference in outcome comparing 180 MEM patients and 363 EM patients treated with oral doxycycline for 14 days. At the 12-month visit, 4/72 (5.6%) MEM patients treated with doxycycline and 5/78 (6.4%) MEM patients in the ceftriaxone group had incomplete response (upper bound of 95% CI 6.4%) while out of 279 EM patients 19 (6.8%) had incomplete response (95% CI for comparison between MEM and EM -6.1 to 4.5%, P=0.91). Different *Borrelia* species (>90% were identified as *B. afzelii*) were similar regarding the proportion of disseminated infection as well as post-Lyme symptoms. The frequency of nonspecific symptoms in patients was comparable to that among controls.

Conclusions The 14-day regimen with oral doxycycline was not inferior to the 14-day regimen with intravenous ceftriaxone for treatment of adult European patients with MEM. Clinical course and long-term outcome of early localized and early disseminated Lyme borreliosis were comparable. Different causative *Borrelia* species had comparable potential to cause disseminated disease as well as unfavorable outcome.

Lyme neuroborreliosis in cases of non-specific neurological symptoms

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Background Analysis of cerebrospinal fluid is required in order to diagnose Lyme neuroborreliosis in the European guidelines. We investigated the symptoms of patients in a highly endemic area who were referred for evaluation of possible Lyme neuroborreliosis, and explored whether cerebrospinal fluid analysis confirmed or ruled out the diagnosis.

Method We reviewed the medical records of all patients who was referred with a question of LNB and underwent lumbar puncture at Sørlandet Hospital Arendal in the period 1 January 2013 to 31 December 2013.

Results A total of 140 patients were referred with suspected Lyme neuroborreliosis. Of these, 110 patients had non-specific neurological symptoms (e.g. fatigue, dizziness and headache), only one of whom received a diagnosis of possible Lyme neuroborreliosis. Thirty patients had symptoms typical of the condition (such as radiculitis or peripheral facial nerve palsy). Six of these were diagnosed with definite Lyme neuroborreliosis, and one with possible Lyme neuroborreliosis. None of those diagnosed with Lyme neuroborreliosis had had symptoms lasting more than six months. 24 % of those assessed for peripheral facial nerve palsy received a diagnosis of either possible or definite Lyme neuroborreliosis.

Interpretation The probability of Lyme neuroborreliosis is low in the absence of typical symptoms of the condition, even when anti-Borrelia antibodies are detected in serum and especially when the symptoms are of long duration. Lyme neuroborreliosis is an important differential diagnosis in cases of newonset peripheral facial nerve palsy in a highly endemic area, irrespective of whether the patient can recall a tick bite or erythema migrans.

Acrodermatitis chronica atrophicans – clinical and laboratory characteristics of 609 Slovenian patients

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Background Information on larger groups of patients with acrodermatitis chronica atrophicans (ACA) is limited.

Methods The retrospective study comprised patients diagnosed with ACA at a single center. The diagnosis was based on three requirements: corresponding clinical findings, presence of borrelial IgG serum antibodies, and skin histology consistent with ACA. Patients' clinical, laboratory and microbiological characteristics were assessed; comparison of findings in two different time periods (1991–2003 vs. 2004–2016) was made.

Results 609 patients (399 females, 210 males; median age 64 years, IQR 55–71), were included. Duration of ACA before diagnosis was 12 (IQR 5–24) months. Skin lesions were located on extremities in all but one patient: 69.3% of patients had lesions on lower extremity, 46.5% on upper extremity, 42.5% bilaterally. Constitutional and local symptoms were reported by 66.2% and 21.8% of patients, respectively. Cerebrospinal fluid (CSF) analysis revealed lymphocytic pleocytosis, elevated protein concentration, and intrathecal synthesis of borrelial IgG antibodies in 4.3%, 26.6%, and 12.0% of patients, respectively. Borreliae (predominantly *Borrelia afzelii*) were isolated from skin, blood, and CSF in 31.5%, 1.1%, and 1.7% of patients, respectively.

Patients assessed in the recent time period were older, had shorter duration of ACA that was more often located on ankle or thigh and less often on shin or upper extremity, had less frequently bilateral involvement, had less often skin atrophy, and less frequently complained of constitutional but more frequently of local symptoms.

Conclusions ACA usually affects older women. CSF abnormalities are rare. Isolation rate of *Borreliae* from skin is about 30%. *B. afzelii* is the most common but not the only causative agent. Patients diagnosed with ACA in the recent time period had shorter duration of skin lesions, had less often skin atrophy, and less often reported constitutional symptoms - probably due to earlier diagnosis.

When to order laboratory testing for the diagnosis of Lyme borreliosis and which test to use?

[Key note lecture]

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Lyme borreliosis (LB) is a tick-borne infection caused by *Borrelia burgdorferi* sensu lato. LB attracts lot of attention in the media which may bring some confusion in clinical diagnosis and choice of diagnotic tests. Currently there are indications of overuse of diagnostic testing for LB with potential implications for patient care and cost effective health management. An overview of diagnostic testing to be used, when Lyme borreliosis is suspected is presented.

The routine method for detecting Lyme borreliosis is serology. Detection of Borrelia specific antibodies has advantages of a high sensitivity and a usefull specificity. The activation of the immune system is central to human survival in general and is the generator of the pathology and clinical manifestations of Lyme borreliosis. The high sensitivity is, however, only obtained if the disease is given a few weeks to develop. High positive predictive values may be obtained if, and only if, clinical evaluation is also considered.

The main recommendations according to current European case definitions for Lyme borreliosis are as follows: Typical erythema migrans should be diagnosed clinically and does not require laboratory testing, the diagnosis of Lyme neuroborreliosis requires laboratory investigation of the spinal fluid including intrathecal antibody production for, and the remaining disease manifestations require testing for antibodies to Borrelia burgdorferi. Testing individuals with non-specific subjective symptoms is not recommended, because of a low positive predictive value. Direct detection of Borrelia DNA may be used in selected cases. At present no other tests are relevant for routine use. A critical approach is needed with due consideration of other diagnostic possibilities. Details will be presented on choice and interpretation of the laboratory results.

Borrelia miyamotoi infection in vitro, in mice and men: understanding its pathogenesis and implications for diagnosis and treatment

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Borrelia miyamotoi is an emerging tick-borne pathogen. In contrast to related *Borrelia* species that cause relapsing fever, it is transmitted by hard-bodied *Ixodes* ticks across the temperate zone of the Northern hemisphere. In immunocompetent patients, infection is characterized by an acute febrile illness, whereas in three immunocompromised individuals from Europe and the USA a chronic meningoencephalitis has been described. Knowledge on the pathogenesis and the clinical course of *B. miyamotoi* is limited, let alone on diagnostic tools and treatment strategies. In recent years, as a collaborative effort, we have studied this emerging tick-borne pathogen in great detail. In this overview talk we present new insights on the clinical course and long-term sequelae from the unique Russian cohort of *B. miyamotoi* disease patients. In addition, we discuss how *B. miyamotoi* evades complement and antibody-mediated killing, based on *in vitro* and *in vivo* murine studies. We have also successfully obtained six clinical *B. miyamotoi* isolates from Russian patients and we will present preliminary WGS data and data on the interaction of these isolates with the human innate and adaptive immune response. From our *in vitro* and *in vivo* murine research novel serodiagnostic markers have emerged, and we describe the dynamics of the antibody response of human *B. miyamotoi* disease patients (and controls) against these antigens. Furthermore, we show data on the seroprevalence in various European cohorts of individuals that have reported a tick bite. Finally, we describe the antibiotic susceptibility of *B. miyamotoi*, including the novel clinical isolates, to the antibiotics that are commonly used to treat Lyme borreliosis and relapsing fever, which could guide future antibiotic treatment strategies.

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Clinical overview of acute Borrelia miyamotoi disease: 239 new cases

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Borrelia miyamotoi is transmitted by *Ixodes* ticks and is the causative agent of a new human disease; the first 50 cases were described in Russia (Platonov et al, 2011). Here we summarize the clinical features of 239 additional *B. miyamotoi* disease (BMD) cases diagnosed between 2010-2016 in Central Russia (Republic of Udmurtia and Sverdlovsk Region, 122 and 117 cases, respectively). Patients developed flu-like symptoms 14 days after tick bite (interquartile range from 11 to 17 days). *B. miyamotoi* DNA was found in acute blood samples at admission in all cases. Median age was about 51-57 years (range from 15 to 88 years) with a preponderance of males (57-64%).

Clinical symptoms consisted of fever (axillary temperature ≥ 38oC), weakness, and headache in nearly all the patients. Erythema migrans was observed only in 2-5% of patients. Myalgia and/or arthralgia was present in 40-75% of patients, nausea in 33-67%, vomiting in 18-43%. More than 60 percent of patients had signs of dysfunction of one or more organs, including the liver (increased ALT and AST in 45-55% of patients), kidney (proteinuria and/or decreased glomerular filtration rate in 25-60% of patients), and heart (myoglobin in blood, abnormal echocardiography). Meningeal signs, with normal cerebrospinal fluid findings, were noted only in 4% of patients. Thrombocytopenia was observed in 43% of patients and 70% had lymphopenia. Recurrent episodes of fever were observed in about 6% of cases with two or three episodes occurring before the start of antibiotic therapy. The above-noted features clearly discriminated BMD and 164 cases of Lyme borreliosis studied for comparison.

Although lethality was absent and most cases of BMD were mild or moderate, the health burden of BMD cases seems to be substantial in Russia.

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Development of a multivalent OspA based vaccine candidate for prevention of Lyme borreliosis

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Lyme borreliosis (LB) is the most common vector-borne disease caused by several genospecies of the spirochete *Borrelia burgdorferi* sensu lato. At present there is no vaccine available to prevent infections. Number of cases is increasing, emphasizing the need for an effective vaccine. In Europe, the majority of LB cases are caused by four different *Borrelia* species expressing six different serotypes (STs) of the Outer surface protein A (OspA, ST1-ST6). In the US, the vast majority of cases are caused by one *Borrelia* species, *B. burgdorferi* (OspA ST1). OspA is one of the dominant surface proteins expressed by the spirochetes when present in the tick midgut and is a proven target for a LB vaccine. An OspA based vaccine (LYMErix) was previously shown to be efficacious against LB in the US. We have developed a multivalent recombinant OspA-based vaccine, VLA15, which targets OspA ST1-ST6. The vaccine includes three fusion proteins, each containing the C-terminal half of two OspA serotypes. In order to increase immunogenicity, the highly purified proteins are triacylated. Immunization with VLA15 formulated with aluminium hydroxide protected mice from a challenge with spirochetes expressing OspA serotype 1, 2, 4, 5 or 6, using infected ticks or *in vitro* grown spirochetes for challenge. Further pre-clinical testing (ELISA, surface binding and growth inhibition) indicated that VLA15 can provide protection against the majority of *Borrelia* species pathogenic for humans, including OspA serotypes 3. A first-in-human Phase 1 clinical study is currently ongoing with the primary objective to investigate the safety of VLA15 in healthy adults <40 years. Furthermore, immunogenicity and dose response of VLA15 will be assessed. Overall 180 subjects are enrolled in 6 treatment groups to receive 12 µg, 48 µg or 90 µg VLA15 with or without aluminium hydroxide. A planned interim data analysis will be performed in Q1 2018.

The long-term outcome of tick-borne encephalitis
[Key note lecture]

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Background Information on long-term outcome of tick-borne encephalitis (TBE) is limited.

Methods Adult patients diagnosed with TBE in the period 2007–2012 and followed-up for 12 months, were invited to an additional visit in 2014, i.e. 2–7 years after TBE, and assessed for the frequency and severity of post-encephalitic syndrome (PES). Patients were asked to refer a person of comparable age without history of TBE to serve as a control.

Results 420/714 (58.8%) patients and 295/420 (70.2%) controls agreed to participate. The proportion of patients with PES was higher at follow-up visit 6 months after acute illness (127/304, 42%, 95% CI: 36–47%) than at 12 months (68/207, 33%, 95% CI: 26–40%), while the proportions at 12 months and 2–7 years after TBE (137/420, 33%, 95% CI: 28–37%) were comparable. Multiple logistic regression revealed that unfavorable outcome at 6 months was associated with cerebrospinal fluid (CSF) leukocyte count ($p=0.030$) and severity of acute illness ($p=0.006$); at 12 months with the disease outcome at 6 months ($p<0.001$); and at the last visit with outcome at 12 months ($p<0.001$). Symptoms, occurring within 4 weeks before the final examination, were more frequent and more constantly present in patients than in controls. The assessment of health related quality of life 2–7 years after acute illness revealed that patients had lower mean scores than controls on physical and mental summary component, but the differences were not significant.

Conclusions The frequency of PES diminishes over time and stabilized 12 months after acute illness, whereas the severity of PES continued to weaken beyond 12 months. Unfavorable outcome at 6 months was significantly associated with CSF leukocyte count and severity of acute illness, at 12 months with the disease outcome at 6 months, and at last visit with disease outcome at 12 months.

Identification of human genes predetermining susceptibility/resistance to tick-borne encephalitis virus

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It is known that the course and outcome of different infectious diseases depends not only on the infectious agent and environmental factors, but also significantly on human genetic factors. However, human genetic predisposition to tick-borne encephalitis (TBE) has been rather poorly studied to date. Two main approaches can be used for identification of human genes that are associated with susceptibility/resistance to TBE virus. A candidate gene approach is based on primary selection of those genes for the analysis that encode proteins with known or presumable antiviral function. Previously, using this approach we analyzed about 70 polymorphic markers (mainly single nucleotide polymorphisms (SNPs)) located in 15 genes and found that 10 SNPs within 6 genes (including the OAS2, OAS3, CD209, TLR3, IL28B, and IL10 genes) are associated with predisposition to TBE (Barkhash et al., 2010, 2012, 2013, 2016). The second approach is based on high-throughput genome sequencing technologies that allow to use a large number of genetic markers distributed along great deal of human chromosomes. In this report, the results concerning a combination of the candidate gene approach and whole exome sequencing method to identification of new TBE predisposition genes in a Russian population are presented. Particularly, we found that the frequency of G allele for non-synonymous rs17576 SNP (A/G, Gln/Arg) in the matrix metallopeptidase 9 (MMP9) gene is significantly higher in TBE patients with severe central nervous system disease (meningo-encephalitis, etc.) (43.5%) as compared with TBE patients with milder meningitis (26.3%), as well as with the population control group (32.5%) ($P = 0.01$ and 0.042, respectively). The work was supported by the Russian Science Foundation (project no. 16-15-00127).

Occurrence and genetic diversity of tick-borne encephalitis and Kemerovo viruses in ixodid ticks of Western Siberia, Russia and Kazakhstan

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Tick-borne encephalitis virus (TBEV), a member of the *Flaviviridae* family, *Flavivirus* genus, and Kemerovo virus (KEMV), a member of the *Reoviridae* family, *Orbivirus* genus, are transmitted by ixodid ticks and can cause acute neurological diseases. In comparison with TBEV, the occurrence and genetic diversity of KEMV in Western Siberia remain poorly studied. KEMV was discovered in 1960s in ticks and patients from Kemerovo province, Russia, and later its investigations were almost suspended. Therefore, the aim of this study was to investigate the prevalence and genetic variability of TBEV and KEMV in ixodid ticks from Western Siberia, Russia and Kazakhstan.

A total of 1958 *Ixodes persulcatus, I. pavlovskyi* ticks and their natural hybrids from Novosibirsk and Omsk provinces, Altai Republic (Russia) and East Kazakhstan province (Kazakhstan) were analyzed for the presence of TBEV and KEMV RNA. TBEV was observed in all studied territories, and KEMV distribution area in Western Siberia was wider than originally thought and included Novosibirsk and Omsk provinces and Altai region in Russia, and East Kazakhstan region arpart from Kemerovo province. The occurrence of KEMV was significantly lower than TBEV in most locations in Western Siberia. TBEV and KEMV were found both in *I. persulcatus* and *I. pavlovskyi* ticks and in their natural hybrids.

TBEV-Sib was observed in all studied territories, in addition, TBEV-Eu was found in Altai Republic. For the first time, TBEV of “886-84” virus group isolated previously only in Eastern Siberia was found in Novosibirsk province. Studied KEMV variants were genetically different from those isolated in the 1960s, which could indicate the ongoing process of evolution of the Kemerovo virus group. Moreover, the possibility of reassortment for KEMV was demonstrated for the first time.

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An unusual case of tick-borne encephalitis in a chamois (Rupicapra rupicapra), Austria, 2017

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Here we report a case of tick-borne encephalitis (TBE) in a chamois (*Rupicapra rupicapra*), which is unusual in several aspects. A hunter shot the 7-8 year old chamois buck on 11 February 2017 at approx. 1,700 metres above sea level in the Bluntau valley, hunting district Oberjoch, which is part of the market town of Golling an der Salzach in the Austrian federal state of Salzburg, about 5 km east of the Austrian-German border. The hunter knew this particular animal because of an old fracture at its left hind leg. The animal showed torticollis and unsteady gait, but was clinically healthy a few days before. No ticks were found attached to the chamois. Pathohistologically the chamois showed a mild non-suppurative leptomeningoencephalitis with perivascular infiltrates consisting of lymphocytes, plasma cells and histiocytes and multiple glial nodes as well as mild acute perivascular hemorrhages. Universal flavivirus RT-PCR on brain samples was positive, and sequencing of the 241 nt long amplicon within the NS5 nonstructural protein gene region revealed 96% identity to Spanish goat encephalitis virus, 91-95% to Louping ill virus, 93-94% to Negishi virus, 92% to Spanish sheep encephalitis virus, ≤ 92% to tick-borne encephalitis virus (TBEV) and 86% to Greek goat encephalitis virus.

The unusual aspects include: acute flavivirus encephalitis in winter; recorded at a high altitude (although the animal could have been infected at a slightly lower altitude); no ticks attached to the animal (which is actually expected in winter); no other virus of the TBE group than the European subtype of TBEV was ever recorded in Austria.

Whole genome sequencing of the chamois-derived TBE group flavivirus is in progress and will be presented at the conference. Also, tick collection in the area is intended in order to gather more information about this novel encephalitic flavivirus in central Europe.

ABSTRACTS POSTER PRESENTATIONS

P1 Genetics aspects of Borrelia miyamotoi

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Studies on the emerging human pathogen, *Borrelia miyamotoi*, are hampered since only several strains were isolated and sequenced to date. We isolated 6 clinical strains from Russian patients with acute *Borrelia miyamotoi* disease (BMD) and performed Whole Genome Sequencing using platforms MiSeq (Illumina Inc., CA, USA) and MinION (Oxford Nanopore Technologies Ltd, UK). This combination of short-read and long-read techniques is new for the investigation of the spirochete's fragmented genomes and may have methodological values by itself. Our approach allowed for the assembly of the complete genomes (chromosome and most unique plasmids) of all isolates.

Based on these data we confirmed the substantial separation of the “Asian" *B. miyamotoi* genotype from the “European" and “American" genotypes and demonstrated genetic variability within the Asian *B. miyamotoi* genotype. However most genes of *B. miyamotoi* belonged to a stable “core genome" and coded proteins with known functions - critical for *B. miyamotoi* survival and infectivity - and unknown functions. Moreover, for each clinical isolate we identified a dominant variable major protein (VMP) gene placed near the promotor at the “expression plasmid", and dozens of other archival VMP genes located on various plasmids.

Using the above data, we have recently started to develop and test a new scheme of *B. miyamotoi* genetic characterization, including optimized and expanded Multi-Locus Sequence Typing of house-keeping genes, as well as sequencing of genes for several important variable immunogenic proteins. Studies are ongoing with the main goal to develop “non-cultural" genetic typing methods. These methods will be used to characterize dozens of already collected Russian *B. miyamotoi* isolates using DNA extracts obtained directly from patients' blood samples and infected ticks.

This study was supported by the grant of Russian Scientific Foundation (project no. 15-15-00072).

P2 Borrelia miyamotoi antigenic proteins and differential expression of its proteins withdrawn

P3 Transovarial transmission rate and filial infection prevalence of Borrelia miyamotoi from Ixodes scapularis collected from hunter harvested white-tailed deer

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Borrelia miyamotoi is a relapsing fever spirochete transmitted by ticks in the *Ixodes ricinus* complex. In the eastern United States, *B. miyamotoi* is transmitted by *I. scapularis,* which also vectors the Lyme disease pathogen. In contrast to Lyme borreliae, *B. miyamotoi* can be transmitted vertically from infected females to their progeny. Therefore, in addition to nymphs and adults, larvae may vector *B. miyamotoi* to humans. Scoles et al. (2001) reported variable filial infection prevalence (FIP) - 6% and 73% - from two vertically infected larval clutches; no other data exist regarding FIP or transovarial transmission (TOT) rates. Thus, we investigated TOT and FIP from engorged females collected from hunter-harvested white-tailed deer in 2015 and 2016 from Wisconsin, New Hampshire, Maine, and Tennessee. After engorged females oviposited, they were tested for *B. miyamotoi* infection. Pools, as well as one hundred individual larvae, were tested from each larval clutch produced by an infected female. In 2015, 4 infected females (n = 205) provided larval clutches resulting in a TOT rate of 75%, and the FIP from two clutches were 92.9% and 100.0%. In 2016, the TOT rate and average FIP were observed as 100.0% and 81.2%, respectively, from 5 infected females (n=188). Altogether, the average TOT rate and FIP from deer-fed females were 88.9% (56.5 - 98.0%; 95% CI) and 84.2% (81.4 - 86.7%; 95% CI) respectively. These data suggest that TOT and FIP are relatively high, but < 100%. Although nymphs pose the greatest epidemiological risk for several *I. scapularis*-borne pathogens, given their abundance and tiny size, larvae may pose the greatest risk for hard tick relapsing fever. Future studies should confirm the generality of these findings within and among *I. ricinus* complex species as well as measure directly the entomological risk index for *B. miyamotoi*.

P4 Investigating Borrelia miyamotoi infection prevalence and density of infected larval, nymphal, and adult Ixodes scapularis in Wisconsin: implications for disease risk

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Borrelia miyamotoi, a relapsing fever group spirochete detected throughout the range of several *Ixodes ricinus* complex ticks, recently has been implicated as a cause of human disease in all Lyme endemic areas. Even though *B. miyamotoi* is transmitted by the same vector tick, and therefore maintained in same enzootic cycle with Lyme borreliae, little is known about its maintenance in nature and the disease risk posed to humans. Vertical transmission of *B. miyamotoi* from infected females to their progeny has been observed, and transmission to wildlife by vertically infected *I. scapularis* nymphs and *I. ricinus* larvae has been demonstrated. Moreover, the first large case series study in the northeastern United States suggested that the annual peak incidence of *B. miyamotoi* human infection might correspond with the peak activity of larval ticks in the northeastern US. Thus, to increase our understanding of *B. miyamotoi* maintenance in nature and the human disease risk, we investigated *B. miyamotoi* infection prevalence in host-seeking larval, nymphal and adult *I. scapularis* and estimated the density of infected ticks per 1000 m2 (DIT). Host-seeking *I. scapularis* were collected from May to October through 2010 to 2012 in Wisconsin in northern central US. We collected 19,249 larvae, 2,761 nymphs and 1,357 adults by dragging and subsets of ticks were tested for *B. miyamotoi* infection by quantitative PCR, resulting in 0.88% larval infection (n=511 pools of 5 larvae), 2.0% nymphal infection (n=782 individual ticks), and 1.0% adult infection (n=730 individual ticks). Estimated DIT of larva, nymph, and adult for *B. miyamtoi* were 0.32, 0.11, and 0.02 (tick/1000 m2), respectively. Larvae, therefore may pose a greater entomological risk for hard tick relapsing fever compared with nymphs and adults. Our observation provides reasonable evidence to reconsider bites from larval *I. scapularis* as a public health threat.

P5 Borrelia miyamotoi, a connection between Lyme disease and relapsing fever?

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Relapsing fever spirochetes are primarily spread through the bite of an infected soft-shell tick [Ornithodoros spp.]. Five relapsing fever spirochetes, including the emerging human pathogen Borrelia miyamotoi, are carried by hard-shell tick species. B. miyamotoi is transmitted by the same Ixodes spp. carrying the causative agents of Lyme disease, species of the B. burgdorferi sensu lato complex in the US, Europe, and Asia. There is no viable, immunocompetent animal model for studying B. miyamotoi in the lab, however, utilizing immunodeficient mice, we have been able to address some basic questions regarding the movement of B. miyamotoi between ticks and mice. Immunocompromised Rag1-/- C57BL/6J, immunocompetent 2-4 week-old C3H/HeN, and immunocompetent 4-6 week-old C3H/HeN mice were infected by intraperitoneal injection of 10^6 B. miyamotoi FR64b, a strain originating from the blood of a small Japanese field mouse. Blood was collected and cultured every 12-hours for 2 weeks. Immunodeficient mice were persistently infected with spirochetes detected in blood 12 hours post infection. Immunocompetent mice in both groups demonstrated detectable spirochetemia 12 hours post infection but rapidly cleared spirochetes. Uninfected larval I. scapularis were fed on infected Rag1-/- mice. PCR showed I. scapularis acquired B. miyamotoi from infected mice. Ticks molted to nymphs and PCR was again performed. B. miyamotoi was detected post-molt, showing no vector specificity, as has been demonstrated with all relapsing fever spirochetes carried by soft-shell ticks. Additional experiments will demonstrate where B. miyamotoi resides within Ixodes as well as time required for transmission and uptake.

P6 Long-term follow-up of patients with Borrelia miyamotoi disease in Russia

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Between 2009 and 2016 we have identified over 300 patients with *Borrelia miyamotoi* disease (BMD) and have described the acute manifestations of BMD elsewhere. As part of the current study, we aimed to describe the long-term effects in a subset of BMD patients.

Russian BMD patients were included in Izhevsk and Yekaterinburg. A total of 41 BMD cases from Izhevsk were subjected to active observation for one year after onset disease. Clinical symptoms were reported, standardized psychological questionnaires were taken, physical- and instrumental examination was performed and blood was drawn for biochemical analysis. In addition, 75 BMD cases from Yekaterinburg were followed during several years and investigated for the presence of anti-GlpQ and anti-VMP IgM and IgG antibodies.

In about 70% of patients no objective medical abnormalities were observed one year after BMD. However, 73% of cases reported subjective complaints like weakness and fatigability (23%), headache (22%), arthralgia (17%), mild motor, cognitive or emotional disturbances (32%). In 20 to 30% of cases an asthenic syndrome and either subjective or objective evidence of cardiac dysfunction (supraventricular extrasystoles, diastolic dysfunction, elevated blood pressure, etc.) were observed. Mild renal dysfunction (albuminuria and decrease of glomerular filtration rate) was found in approximately 30%. Whether this is higher than in the local population remains to be investigated. Finally, one or more years after BMD the specific anti-*B. miyamotoi* antibodies were usually absent or significantly lower than in the acute phase of BMD.

In conclusion, we did not find evidence of chronic or re-activated *B. miyamotoi* infection, or long-term sequelae specific for BMD. However, quality of life was decreased in some patients with a history of BMD as far as persisting symptoms and signs of (mild) organ dysfunction were found on follow up.

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P7 In vitro susceptibility of the relapsing fever spirochete Borrelia miyamotoi to antimicrobial agents

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Borrelia miyamotoi disease (BMD) is an emerging tick-borne disease found across the temperate zone. Treatment of BMD is empirically based on treatment of Lyme borreliosis and antimicrobial susceptibility of *B. miyamotoi* has not been studied. We therefor set out to determine the *in vitro* antimicrobial susceptibility of six recently isolated clinical *B. miyamotoi* strains, two *B. miyamotoi* laboratory strains (HT31 and LB-2001) isolated from ticks, and, for comparison, one relapsing fever *Borrelia* (*B. hermsii* HS1) and two Lyme *Borrelia* strains (PKo and N40).

A standardized 96-well microdilution method was used, testing a dilution series of amoxicillin, doxycycline, azithromycin and ceftriaxone. MICs were determined through colorimetric change and microscopy.

B. miyamotoi clinical isolates and labstrains were susceptible to ceftriaxone and azithromycin, with MICs comparable to MICs for Lyme *Borrelia* and *B. hermsii* controls strains. Good susceptibility to doxycycline was found for all tested *B. miyamotoi* and *B. hermsii,* with MICs ranging 0,125 to 0,5 mg/L, which was lower than the MICs of the tested Lyme *Borrelia* controls (2-4 mg/L). The MICs for amoxicillin of all clinical *B. miyamotoi* isolates and labstrains and *B. hermsii* - ranging from 16 to 32 mg/L - were above the clinical breakpoint for resistance (4 mg/L). In contrast, MICs from two tested Lyme *Borrelia* controls were at this breakpoint or below.

Our data suggests that doxycycline, azithromycin and ceftriaxone can be used for treatment of BMD. Oral amoxicillin is currently used in the treatment of borrelial infection. Although in vitro susceptibility does not necessarily reflect in vivo effectiveness, since we found antimicrobial resistance of all tested *B. miyamotoi* strains to amoxicillin in vitro, this finding warrants further study.

Part of this study was supported by a grant of the Russian Scientific Foundation (project no. 15-15-00072) to Russian co-authors.

P8 Detection of anti-Borrelia miyamotoi antibodies in retrospective and prospective cohorts

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Introduction: *Borrelia miyamotoi* is found in 0.5-4% of *Ixodes ricinus* ticks in Europe and is the causative agent of hard tick-borne relapsing fever (HTBRF). The first human cases were found in Russia in 2011 and subsequently in Europe, the US and Asia by experimental molecular and serological tests. These patients presented with an acute febrile illness, and three similar immunocompromised cases were described with a meningoencephalitis due to *B. miyamotoi*. However, the extent of human exposure to *B. miyamotoi* after a tick-bite in Western-Europe remains unknown. During infection with *B. miyamotoi*, antibodies against several antigens, including the variable major proteins (Vmps) and glycerophosphodiester phosphodiesterase (GlpQ), develop in humans. We here aim to estimate the exposure to *B. miyamotoi* after a tick-bitein Western-Europe by detecting Vmp- and GlpQ-specific antibodies.

Methods: Several cohorts containing acute and convalescent sera of individuals from Western-Europe bitten by ticks are available for serological testing. Detailed clinical information, e.g. history of fever or previous *Borrelia burgdorferi* exposure, is also available. Detection of *B. miyamotoi* antibodies will be performed byELISAs and Western blots using several recombinant Vmps and GlpQ as antigens. Healthy blood donors from the same geographical location represent the control groups.

Results: Recombinant proteins for ELISA and Western blot have been generated, experimental ELISA and Western blot protocols have been optimized, and sera from a Dutch and Swedish tick-bite cohort have been obtained. At the meeting we will be able to share our preliminary data on IgM and IgG antibodies against Vmps and GlpQ in acute and convalescent sera from these patient cohorts.In the near future a prospective study will be initiated to follow-up patients who develop fever after a tick-bite.

Conclusion: We strive to estimate exposure to the emerging hard tick-borne pathogen *B. miyamotoi* and the incidence of HTBRF in Western-Europe.

P9 Borrelia miyamotoi infection in Austria suggested by antibodies to glycerophosphoryl diester phosphodiesterase

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The spirochete *Borrelia miyamotoi* is closely related to the group of relapsing feverborreliae. It is, however, vectored by ixodid ticks just as the agents of Lyme borreliosis. Initially receiving little attention, evidence renders *B. miyamotoi* a human pathogen. Several studies report a flu-like illness with a wide variety of symptoms comprising relapsing-fever-like episodes, fatigue, headache, myalgia, arthralgia and others. Diagnosis of the disease is challenging and currently mainly based on serology and/or detection of specific DNA by polymerase chain reaction. We recently reported the presence of this spirochete in *Ixodes ricinus* ticks in Austria.

In the present study immunogenic glycerophosphoryl diester phosphodiesterase (GlpQ) protein of *B. miyamotoi* was recombinantly expressed and purified. It was used as antigen in immunoblotting for the detection of specific antibodies.

We tested sera of a group of persons who were regularly exposed to ticks, namely hunters, and of persons with proven tick bites, namely patients with confirmed Lyme neuroborreliosis. Moreover, we tested sera of patients with high titers of IgG antibodies against *B. burgdorferi* sensu lato (Bbsl).

We detected anti-GlpQ antibodies in 2/53 (3.77%) sera of hunters, and in 1/11 (9.09%) serum of a Lyme borreliosis patient. In the group with high titers of IgG antibodies against Bbsl 17/74 (23%) tested positive.

Our preliminary results show that infections with *B. miyamotoi* presumably also occur in Austria but were not yet recognized as disease. Additionally, the findings suggest frequent co-transmission of Bbsl and *B. miyamotoi*. Further studies are projected in order to develop most advanced and effective diagnostic tools.

P10 Antibody responses to GlpQ and variable major proteins in patients with Borrelia miyamotoi disease

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Borrelia miyamotoi disease (BMD) is an emerging *Ixodes* tick-borne disease described in North-America, Europe, Russia and Asia. Serodiagnosis by glycerophosphodiester-phosphodiesterase (GlpQ) - present in *B. miyamotoi,* but not in *Borrelia burgdorferi* sensu lato (the causative agent of Lyme borreliosis) - is experimentally performed. We have previously shown that by switching between variable major proteins (Vmps), *B. miyamotoi* is able to evade humoral immunity and that Vmps are antigenic in humans. This suggested that Vmps are interesting targets as serodiagnostic markers in BMD.

Our aim was to study the antibody response against GlpQ and Vmps in patients with BMD. We included a unique cohort of 50 Russian BMD patients, sampled at multiple timepoints. Healthy blood donors and patient controls were also included. All samples were tested for antibodies by ELISA, positive sera were confirmed using Western Blot.

We show - for! the first time - dynamics of specific IgM and IgG antibodies in BMD. IgM antibodies peaked 11-20 days after onset disease and IgG between 21-50 days. IgG, unlike IgM antibodies, were still increased after one year. Moreover we show the serodiagnostic potential of individual seromarkers and their combinations. Interestingly – from 44 patients of whom sera were available at least 11 days after onset disease - 43 were positive (97,8% [95% CI: 87,1-100]) using IgM or IgG antibodies against both GlpQ and any Vmp, while we found a specificity of 100,0% (95% CI: 97,8-100) for IgM and 98,3% (95% CI: 95,2-99,6) for IgG.

Our findings show the great potential of these antigens as serodiagnostic markers, facilitate future epidemiological and clinical studies on BMD and could lead to the development of a diagnostic test that can be used in daily clinical practice.

Part of this study was supported by a grant of the Russian Science Foundation (project 15-15-00072) to Russian co-authors.

P11 Antibody response in Borrelia miyamotoi infection studied by plane protein microarray

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During *Borrelia miyamotoi* disease (BMD) patients produce antibodies to glycerophosphodiester-phosphodiesterase (GlpQ) and to highly antigenic surface variable major proteins (Vmps). Individual *B.miyamotoi* spirochetes expresses presumably one VMP, but *in vivo* "switching" between Vmps may occur, as observed in animal models of *B. miyamotoi*.

We here studied IgM and IgG antibody responses by producing a plane protein microarray containing GlpQ and four Vmps: variable small protein (Vsp) 1, variable large protein (Vlp) 15/16 (Delta subfamily), Vlp5 (Gamma subfamily), Vlp18 (Alpha subfamily). This selection of Vmps represents all subfamilies of *B.miyamotoi* Vmps and these specific VMPs were selected based on expression in *B.miyamotoi* strains HT31 or LB-2001. In addition our microarray contains 14 antigenic variants of 8 *B.burgdorferi sensu lato* proteins.

We tested 219 sera from 50 PCR-confirmed Russian BMD patients. Samples were drawn between hospital admission and one year after disease. In addition, we tested 70 sera from 70 healthy blood donors. Antibodies to GlpQ were detected in 46/50 BMD cases and 50/50 BMD patients produced antibodies to at least one Vmp: IgM and/or IgG antibodies to Vlp15/16, Vsp1, Vlp5 and Vlp18 were detected in 34, 22, 13, and 11 patients, respectively. Interestingly, antibodies to two different Vmps were detected in blood of 13 patients, antibodies to 3 or 4 Vmps – in blood of 4 and 3 patients, respectively.

IgM antibodies peaked approximately 13 days after onset disease and decreased one year after; IgG antibodies reached a plateau at day 40 that remained year after.

Serial measurement of anti-GlpQ and anti-Vmp antibodies provided the possibility to diagnose BMD with 99% specificity and 92% sensitivity. Since about 40% of patients responded to two of more Vmps, this suggests antigen switching and immune evasion in BMD.

This study was supported by the grant of Russian Scientific Foundation (project 15-15-281 00072).

P12 Structure of CRISPR / Cas-systems in the genome of the strain Borrelia miyamotoi LB -2001, revealed by bioinformatics methods

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CRISPR / Cas-system of bacteria is defined as a system for protecting bacteria from phages and plasmids. Methods of bioinformatics can reveal their loci and structures in the genomes of bacteria. We present the results of the search and analysis of CRISPR / Cas systems in the genome of the *Borrelia miyamotoi* LB-2001 strain (GenBank No. CP006647). To search for CRISPR / Cas- systems, the software modeling methods MacSyFinder ver 1.0.2 was used. The search for cas-genes was carried out using programs from packages makeblastdb ver.2.2.28 and HMMER ver.3.0. For the search and analysis of CRISPR-cassettes in bacterial genomes, we developed an algorithm of 5 programs: 1) PILER-CR; 2) CRISPI; 3) CRISPRFinder; 4) CRT; 5) CRISPRDetect. Also, following programs were used to search for phage associates detected through the spacers of CRISPR cassettes: BLASTn and CRISPRTarget, Mycobacteriophage Database and Phages database. Only one type of cas-genes, csf4_TypeU, was detected. In the genome, two CRISPR cassettes were also found. In the first CRISPR cassette, 8 spacer sites were identified, separated by 9 nb repeats. The second CRISPR cassette had 6 spacers also separated by 9 nb repeats. Through the spacers of the first CRISPR cassette, complementary sites (protospacers)of phages with a size of 13 to 25 nb relating to bacterial genera *Mycobacterium, Arthrobacter, Rhodococcus,* Gordonia were identified. Through the spacers of the second CRISPR cassette, protospisers of phages 14 to 22 nb relating to the bacterial genera *Arthrobacter, Streptomyces, Rhodococcus,* Mycobacterium were identified. Thus, the bioinformational programs used make it possible to search for the structures of CRISPR / Cas systems in the bacterial genomes, and also through the spacers of their CRISPR cassette to identify the phagotypes with which this bacterium have met in its evolutionary history.

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P13 Distribution and tick-borne pathogens of Ixodidae ticks in the Northeastern and Northern Altai (Republic of Altay, Russia)

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Tick-borne pathogens are wide spread all over the world and pose various risks for human health. So, the aim of this study was to get a current overview of the biodiversity and spread of those pathogens in ticks at different sites of Altai (Republic of Altay, Russia). To contribute to the knowledge of pathogen status we collected ticks in the Northeastern Altai and Northern Altai, in April–Jule, 2016 by flagging. Ticks were identified down to species level. The flagging technic represented a high range of vectors, mainly Ixodes persulcatus, Ixodes pavlovskyi, Haemaphysalis concinna, and Dermacentor silvarum. In total 700 ticks were collected. I. persulcatus were found in all examined sites. Three other species of ticks were found in Northern Altai. A total of 470 questing I. persulcatus ticks were individually analysed by polymerase chain reaction. If positive on bacteria's and viruses DNA, samples were sequenced. We identified Borrelia burgdorferi, B. miyamotoi, Anaplasma phagocytophilum, Ehrlichia muris, Babesia microti, B. venatorum, Tick-borne encephalitis virus (TBEV), and Kemerovo virus. Three hundred three ticks (64.5%) were positive for tick-borne pathogens. Ninety-nine (41.7%) and twenty-three (4.9%) specimens tested positives for B. burgdorferi and B. miyamotoi respectively. A. phagocytophilum and E. muris were detected in nineteen (4%) and forty-two(8.9%) specimens respectively. Eighteen ticks (3.8%) was found positive for B. microti and B. venatorum. TBEV and Kemerovo virus were detected in 3 and 2 specimens respectively. This biodiversity should be considered in diagnostics of tick-borne diseases in human medicine in Republic of Altay.

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P14 The unique combined natural foci of tick-borne infections in the territory of Ekhirit-Bulagatsky district in Irkutsk region, Russia

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The combination of the variety of nature and climatic conditions in the territory of Ekhirit-Bulagatsky district in Irkutsk region (Eastern Siberia of Russia) (coniferous and mixed forests, forest-steppe and steppe, meadows and marshes) leads to the constant presence of 4 ticks species (*Ixodes persulcatus*, *Dermacentor silvarum*, *D. nuttalli* and *Haemaphysalis concinna*) known as vectors for transmissible infections pathogens, as well as their feeders (pathogens reservoirs). The main reservoir hosts found here are *Spermophilus undulatus*, *Microtus gregalis*, *M. oeconomus*, *M. arvalis*, *Myodes rutilus* and others.

The diversity of landscapes biocenotic structure gives the possibility to circulate for wide range of genetically diverse pathogens transmitted by Ixodid ticks in this area. Also, a unique feature of the area is the presence on its territory the natural foci where the joint circulation of the following pathogens: tick-borne encephalitis virus (TBEV), *Borrelia miyamotoi*, *Rickettsia sibirica*, *R. raoultii (DnS28)*, *Anaplasma phagocytophilum*, *Ehrlichia muris*, *Francisella tularensis*, *Babesia* spp. was observed.

The area of Ekhirit-Bulagat district is characterized by significant genetic variability of microorganisms and viruses found there. The circulation of three TBEV subtypes - Far Eastern, Western (European) and Siberian, - was discovered. In addition, the TBEV strains with unique genetic structure (possible new TBEV subtypes, strains 886-84 and 178-79) were found there.

Based on the analysis of 16S rRNA gene and groESL operon nucleotide sequences the samples of *Anaplasma phagocytophilum* found in *Ixodes persulcatus* ticks were divided into two genetic groups.

Based on the analysis of 18S rRNA gene sequences in *Ixodes persulcatus* and *Haemaphysalis concinna* ticks samples collected in the territory of Ekhirit-Bulagatsky district Babesia phylogenetically similar to piroplasms of small ruminants (*B. crassa* and *B. motasi*) were found.

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P15 Tick-borne infections in natural foci of the Baikal region of Russia (research in 2015-2016)

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Circulation of tick-borne infections was analyzed in natural foci of the Baikal region (Irkutsk Region, Republic of Buryatia) using the Real-Time PCR method. The Baikal region is a highly endemic region with regard to tick-borne infections, especially tick-borne viral encephalitis. 1015 ticks were collected in spring-summer epidemic seasons 2015-2016 by the method of field collection of material. 513 females were selected among the collected ticks for analysis. Irkutsk district, Angarsk district and Slyudyansky district were represented in Irkutsk region. In the Republic of Buryatia Kurumkansky district, Barguzinsky district, Tunkinsky district and peninsula "Svyatoy Nos" were presented. DNA and RNA of tick-borne pathogens (TBEV, *Ehrlichia spp., Anaplasma spp., Borellia spp*.) were detected by the Real-Time PCR method. The tick-borne encephalitis virus was detected in 21 cases. The highest virus-resistance was registered among the ticks collected in the Barguzinsky district ("Adamovo") 8.6% and on the peninsula Svyatoi Nos ("Kurbulik") 9.7%. Virus-resistance of ticks in these areas exceed the average in Russia by 4-5 times. *Borrelia spp*. was found in 225 cases, *Anaplasma spp*. in 42 cases and *Erlichia spp.* in 57 cases. The most unfavorable in Borrelia was Slyudyansky district (76%). In addition, a mixed infection (TBEV + Borrelia) was detected in 6 cases. The most "prosperous" district of the Baikal region in Borrelia was Kurumkansky district (22.2%).

The obtained data show the actual epidemiological situation with regard to tick-borne infections in the Baikal region. This information will be used to further assess the state of natural foci of tick-borne infections in the region in order to develop the most effective strategy for specific and nonspecific prevention of tick-borne infections in southern Siberia.

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P16 Detection of Anaplasma phagocytophilum and other pathogens in wild animals and their ectoparasites from Czech Republic

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Vector-borne infections increasingly a public health problem in Europe. The aim of this study was to assess the prevalence of vector-borne pathogens in deer and their ectoparasites from the Krkonoše National Park and in wild boar from a hunting area in Moravia.

A total of 67 samples of red deer blood, 99 samples of wild boar spleen, 43 ticks *Ixodes ricinus* (18 fed female, 18 unfed female, 7 male) and 42 keds *Lipoptena cervi* from 71 red deer and roe deer were investigated during the year 2014-2016. All the 251 samples were screened for *Anaplasma, Borrelia, Rickettsia* and *Babesia* by nested PCR and by real-time PCR with assay for *msp2*, *ospA*, *gltA*, 18S rRNA. All positive samples were identified by direct sequence analysis.

Six wild boar (6.1%) were positive for *A. phagocytophilum*. We did not find another infection in wild boar. Four red deer (5.9%) and three red deer (4,5%) were positive for *A. phagocytophilum* and *Babesia sp.*, respectively. Ticks collected from red deer and roe deer were highly infected by *A. phagocytophilum* (9 ticks, 20.9%) and *Rickettsia* spp. (6 ticks, 14%). One tick (2.3%) was positive for *B. burgdorferi* s. l. We found a comparatively lower level of infection of *A. phagocytophilum* in keds (3 keds, 7.1%). Only 1 ked (2.4%) was infected by *B. burgdorferi* s. l.

Our study shows that wild boar and deer might be involved in the natural transmission cycle of *A. phagocytophilum* and *Babesia* spp. Ticks and keds were highly infected by *A. phagocytophilum* and *Rickettsia* spp.; they might play an important role in maintenance cycle of *Rickettsiaceae.*

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

P17 Prevalence of tick-borne pathogens in questing ticks Ixodes ricinus in urban area of Prague, Czech Republic

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The most important vector of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia* spp. and *Babesia* spp. is the hard tick *Ixodes ricinus* associated with deciduous and mixed forests. Large tick populations have been observed in European city green areas. The aim of this study is to assess the prevalence of these pathogens in questing ticks in urban areas in the city of Prague.

We monitored four different public parks with deciduous or mixed forests, brooks, ponds and wetlands. In these parks there are known reservoirs and tick-maintenance hosts of tick-borne pathogens.The parks are also frequented by companion animals like dogs and cats. Ticks were collected by flagging vegetation in spring 2014 and 2015. The species and the development stage of the ticks were determined. All the samples were screened for pathogens by PCR and positive samples were identified by direct sequence analysis.

A total of 675 *I.ricinus* ticks were collected (128-females, 160-males, 387-nymphs). Two-hundred-twenty-one ticks (32.7%) were found to be infected by *B.burgdorferi* s.l., the most infected were males (33.8%) and nymphs (34.9%). *B.garinii* was the most frequent genospecies in ticks (49.8%). We also detected *B.afzelii* (26.2%), *B.bavariensis* (7.2%), *B.burgdorferi* s.s. (3.6%), *B. spielmanii* (0.5%). Fourty-seven ticks (7%) were infected by *A.phagocytophilum*. The most infected were males (13.8%). Twenty ticks (3%) were infected by *Rickettsia* spp., the most infected were males (4.4%). Fourteen ticks (2.1%) were infected by *Babesia* spp. We found 39.6% positive ticks. Twenty-six ticks (3.9%) were infected by two pathogens.

These findings show higher *Borrelia* and *Anaplasma* prevalence in ticks than in other studies from central Europe. Monitoring of these diseases in ticks is important because it enables us to estimate the disease risk for humans in these locations due to the exposure of both humans and their domesticated animals to potentially infected ticks.

P18 Europe-wide meta-analysis of Borrelia burgdorferi sensu lato prevalence in questing Ixodes ricinus ticks

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Lyme borreliosis is the most common zoonotic disease transmitted by ticks in Europe and North America. Despite having multiple tick vectors, the causative agent *Borrelia burgdorferi* sensu lato, is vectored mainly by *Ixodes ricinus* in Europe. In the present study, we aimed to review and summarize the existing data published from 2010 to 2016 concerning the prevalence of *B. burgdorferi* s.l. spirochetes in questing *I. ricinus*. The prevalence of *B. burgdorferi* s.l. spirochetes in ticks has been considered one of the most crucial elements of risk assessment for Lyme borreliosis. The primary focus was to evaluate the infection rate of these bacteria in ticks, accounting for tick stage, adult tick gender, region and detection method, as well as to investigate any changes in prevalence over time. We compared the new results with previously published data in order to evaluate any changing trends in tick infection. The literature search identified data from 24 countries with 115,028 ticks in total inspected for infection with *B. burgdorferi* s.l. We showed that the infection rate was significantly higher in adults than in nymphs and in females than in males. We found significant differences between European regions, with the highest infection rates in Central Europe. The most common genospecies were *B. afzelii* and *B. garinii*, despite a negative correlation of their prevalence rates. There were no statistically significant differences found among the prevalence rates determined by conventional polymerase chain reaction (PCR), nested PCR or real-time PCR.

P19 Species and subspecies diversity of Borrelia burgdorferi sensu lato strains from Serbia

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Borrelia burgdorferi sensu lato complex (Bbsl) is a group of at least 21 species of tick-borne spirochetes that cause Lyme borreliosis (LB) in Europe and North America. At least five species *B. afzelii*, *B. garinii*, *B. bavariensis, B. burgdorferi* sensu stricto and *B. spielmanii* cause LB in humans, but *B. lusitaniae, B. valaisiana,* and *B. bissettii* also have been linked to human LB. The geographic distribution of Bbsl in Europe shows dynamic spatial and temporal variations. Since different *Borrelia s*pecies usually correlate with a different clinical manifestation of LB, knowledge of the geographic distribution of the pathogen is very important for understanding the ecology and epidemiology of the disease. The aim of the study was to analyse Bbsl strains isolated from *Ixodes ricinus* ticks from Serbia by pulsed-field gel electrophoresis after *Mlu*I restriction of the genomic DNA (*Mlu*I-LRFP). Molecular typing of 28 representative Serbian Bbsl strains with *Mlu*I-LRFP method revealed 4 species: *B. lusitaniae* (8/28; 28.6%), *B. afzelii* (13/28; 46.4%), *B. garinii* (5/28; 17.9%), *B. valaisiana* (2/28; 7.1%). These results were in accordance with previous ones obtained with RT- PCR for *hbb* gene, and *Mse*I and *Dra*I RFLP of 5S-23S intergenic spacies. *Mlu*I-LRFP method revealed high level of diversity on subspecies level. *B. lusitaniae* strains were delineated in four types (Mll2, Mll3, Mll4, Mll5), from which Mll types 3, 4 and 5 were described as new. *B. afzelii* strains were separated into two Mla types (Mla1 and Mla2), as well as *B. garinii* (Mlg1 and Mlg2) and *B. valaisiana* (Mlv1 and Mlv2). High diversity on subspecies level of Serbian Bbsl presents a tool for monitoring Bbsl geographic distribution and could influence on pathogenic potential of local strains.

P20 Cohabitation of Borrelia spp. and Rickettsia spp. in ticks on the south coast of New South Wales, Australia withdrawn

P21 Ixodes ricinus infestation in cattle and goats: host preference in a grazing herd in a mountain pasture (northern Italy)

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Breeding of dairy cattle and goats in alpine environment is often characterized, in spring-summer, by extensive grazing. In the same period, in northern Italy, density of questing *Ixodes ricinus*, the main vector of *Borrelia burgdorferi* s. l. in Western Europe, peaks. Grazing ruminants, particularly wild cervids, contribute to maintenance and spreading of ticks. Aim of this study was to determine *I. ricinus* host preferences in a herd composed by both cattle and goats at pasture. Ticks were monthly collected from April throughout September on 26 randomly selected animals (11 cattle, 15 goats) of a mixed herd (H1) free to graze in a large mountain area (~200 ha) in northern Italy (Varese province, Veddasca Municipality 46°04'13"N, 8°47'33"E). In April ticks were also collected on animals of a second herd (H2), exclusively composed by goats, grazing in Veddasca Valley (Varese province, Curiglia Municipality, 43°03'39"N, 8°48'17"E). The two pastures were less than 2 km away in straight line, their altitude was similar (900 m a.s.l.) as well as the vegetation. In April, 178 *I. ricinus* (137 female, 41 male; 4.2 ticks/animal) were collected from 42 animals. Average abundance of infestation was 8.5, 1.6 and 4.2 ticks/animal in cattle from H1 (C_H1), goat from H1 (G_H1) and goat from H2 (G_H2) respectively. Abundance of infestation in the three groups was compared by Kruskal-Wallis test; difference of abundance was highly significant (p-value<0.001). Pairwise comparisons showed that both G_H2 and C_H1 presented significant higher abundance than G_H1, while G_H2 and C_H1 didn't differ. Apparently, when cattle and goats graze together, *I. ricinus* display host preference for cattle, but when goats graze alone they present higher abundance of infestation. Differences between goats and cattle in H1 were observed also in late spring and summer, when abundance of infection increase.

P22 Translating TekenNet results into tick bite prevention policy advice in Belgium: first steps and future perspectives

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Tick-bite surveillance in Belgium occurs through TekenNet, a citizen-based platform. Besides its usage as an online information tool with readily available information on tick bite occurrence, its surveillance results need to be translated into advice for tick bite primary prevention, tailored to the situation in Belgium and its different regions. The TekenNet platform also serves as a helpdesk by receiving many questions on ticks and tick-borne diseases. A frequency analysis of question topics allows identifying clear needs and information gaps in the general public.

Analyses based on the TekenNet surveillance in 2016 provide a first insight on tick bite occurrence in Belgium. Tick bites were reported throughout the year, but the highest numbers were recorded from May to August. In 2016, Leisure activities were reported as prominent for tick bite exposure (87.8%). Most individuals reported to have acquired their tick bites in the garden (44.6%) or in forest (37.0%) and within a short distance of their residence (31.4% less than 1km, 66.7% less than 5km radius). With regard to questions received by the TekenNet platform, examples of top ranked topics were: repellents and feasible or effective measures for tick protection in the human - pet interface, how to handle high tick abundance and Lyme borreliosis diagnostics and specialist contacts.

In line with published findings in neighbouring countries, these results suggest that besides investing in preventive measures in forested environment, advanced communication on personal protection measures, ecological tick control or spatial restriction strategies in private gardens would be of high value for prevention of tick bites in Belgium. In the coming years the TekenNet platform aims at assisting targeted tick bite prevention efforts through the development of focused risk maps and predictive temporal models.

P23 TekenNet: an online citizen-based platform for the reporting of tick bites in Belgium

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Ticks are important vectors of infectious diseases affecting human and animal health worldwide. In order to investigate and map the risk of tick bites in Belgium, an online citizen-based platform (TekenNet) was launched in June 2015. Citizens can report tick bites through a webpage and an application for mobile phones. A short questionnaire allows the collection of additional data such as location of residence and tick bite occurrence, type of environment and activity. In addition to anonymous reporting (individually and as a group), a cohort of permanent users has been constituted. The records of this group (including zero-reporting) will provide a baseline for future modelling, since they are less influenced by external factors such as temporary increased media attention. These permanent users are also asked to report occurrence of erythema migrans.

The reported bites are presented on a map of Belgium to provide more accurate information on preceding and current tick activity and the spatial distribution of human tick bite occurrence in the country. Based on the TekenNet results, tick bites are shown to be widely distributed over Belgium, with higher incidences in the provinces with the most suitable habitat for ticks and reservoir hosts (Luxemburg, Brabant, Limburg and Namur).

In addition to mapping the risk of tick bites, the TekenNet platform is a useful tool to inform citizens on Lyme borreliosis and preventive measures to avoid tick bites. In 2017, it also serves to constitute a large and geographically comprehensive collection of ticks for pathogen screening, where citizens are asked to send ticks via postal mail.

P24 Germany, 2012: how an extreme weather spell in the winter can influence subsequent vector tick abundance and tick-borne disease incidence

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Natural conditions are always complex with a multitude of different biotic and abiotic factors acting on organisms all the year round. Extreme weather spells may offer the rare opportunity to learn about the influence of certain weather factors on populations, in this case *Ixodes ricinus* ticks. There was an extreme cold spell in Germany from late January to mid-February 2012 with near-ground temperatures constantly below 0°C for at least 15 days and often dropping to a range of −15 °C to −20 °C or even lower at night. In the tick season after this cold spell, the mean host-seeking activity of *Ixodes ricinus* nymphs was significantly lower at 4 out of 4 (field-plot method) and 3 out of 4 (flag method) German forest locations, respectively, when compared to that in 2011. The decline of *I. ricinus* host-seeking activity from 2011 to 2012 was especially distinct in the absence of any snow cover during the cold spell providing strong evidence that the snow cover acted as an effective buffer, protecting *I. ricinus* ticks from low temperatures in the subjacent leaf litter. There was also a significant correlation between the local minimum snow depth during the cold spell and the decrease in the local numbers of tick-borne encephalitis cases in different southern German administrative districts in 2012 compared with 2011. This provides additional indirect evidence that the decrease in tick abundance was particularly strong in those areas with limited snow depth during the cold spell.

P25 Altered gene expression upon infection with Borrelia afzelii in nymphal Ixodes ricinus salivary glands during feeding withdrawn

P26 DNA-binding proteins SpoVG and PlzA are important regulators for Borrelia burgdorferi to adapt to stress

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In order to survive in two very different types of hosts, *Borrelia burgdorferi* must be able to sense its current environment, and respond by appropriately controlling its cellular processes at each step in the infectious cycle. We are investigating the mechanisms by which *B. burgdorferi* regulates gene and protein expression in response to environmental cues, particularly those associated with the tick. Understanding how *B. burgdorferi* within the tick is able to adapt in a way that ensures survival can identify key targets for new antibiotic therapies. The borrelial proteins SpoVG and PlzA (which binds cyclic-di-GMP) are highly expressed in the tick, but not in the mammal. We discovered that both proteins bind to specific DNA sites throughout the genome, often with evidence of cooperativity, and affect expression of numerous genes. Known binding sites include the promoters of both *spoVG* and *plzA* (thus regulating their own expression), the promoter and constant region of *vlsE* (the antigenically variable surface protein), and the promoters of *glpF* and *glpD* (which metabolize glycerol-a necessary carbon source for *B. burgdorferi* in the tick). SpoVG also binds to RNA, which can further affect protein levels by modulating mRNA translation. We demonstrated that these two proteins serve as important regulators for *B. burgdorferi* to adapt to stressful culture conditions, and hypothesize that they function in a similar manner to permit bacterial survival in the tick. Ongoing studies are focused on elucidating what cues SpoVG and PlzA respond to, and how they permit *B. burgdorferi* to adapt, and survive within the tick midgut.

P27 The role of glutathione metabolism in host defence against Borrelia burgdorferi

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The host response against *Borrelia burgdorferi* plays a crucial role in both the initiation as well as the outcome of Lyme disease. Previously, we have shown that pathogen-induced changes in glucose metabolism are important for the immune response against *Borrelia*. To elaborate on this, we aimed to identify other metabolic pathways specifically affected by *Borrelia*-infection and to determine their role in host defense against the pathogen. First, metabolome analysis was performed on monocytes from healthy volunteers stimulated with *Borrelia burgdorferi* compared to other stimuli. Second, metabolome analysis was performed on serum samples from acute Lyme disease patients versus serum samples from patients with other bacterial infections. Interestingly, in both analyses, glutathione metabolism was shown to be one of the pathways most significantly affected by *Borrelia* infection. In addition, these changes appeared to be specific to *Borrelia* as they were not seen in the other infections analyzed. The metabolome findings were supported by previously published transcriptome data, showing persistent changes in gene expression of glutathione-related genes in Lyme disease patients. Additional *in vitro* validation experiments were performed to determine the role of glutathione metabolism in the inflammatory response against *Borrelia.* All in all, these data point towards a central role for glutathione in host defense against *Borrelia* infection. As glutathione is known to exert many protective functions, by regulating redox metabolism and functioning as a detoxifying agent, these findings may contribute to elucidating the pathogenesis of Lyme disease and help explain the variability in disease outcome. In addition, the identification of a pathogen-specific metabolic profile may contribute to improved diagnostics of Lyme disease.

P28 Genomics of two sister species to understand host association in Lyme Borreliosis: Borrelia bavariensis and B. garinii

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According to our current knowledge, *Borrelia bavariensis* and *B. garinii* are the taxonomically closest species in the *Borrelia burgdorferi* sensu lato species complex in Eurasia that do not share the same reservoir hosts. Indeed, both species are human pathogens and are widespread in Eurasia, but *B. bavariensis* uses rodents as reservoir hosts whereas *B. garinii* is adapted to birds. These two species thus form an ideal system to look for genetic factors involved in host association and to understand how *Borrelia* can switch to a new host type. Combining data from Pacific Bioscience long-read sequencing with Illumina MiSeq data, we describe the full genomes of about 30 strains for each species and reconstruct their evolutionary history. Whereas the main chromosome of both species show low divergence, their plasmid contents differ and we could identify plasmids that were specific to one species and are thus good candidates for playing a role in host association. We identified several recombination events and plasmid rearrangements within and between species and hypothesize that host switches may be facilitated by recombination events of genes/plasmids conferring adaptation to a new host type. Finally we analyzed sequences of the so-called CRASP (for Complement Resistance Acquiring Surface Proteins) known to be associated with human complement resistance and identified non-synonymous divergences between the two species that could be involved in evading the innate immune system of different hosts. We conclude that these two sister species represent an ideal system for the study of host association in *Borrelia* and propose further studies including *Borrelia* transformation and functional genetics to unravel the evolution of host association in these two, and potentially other, *Borrelia* species.

P29 Genotyping of Borrelia garinii strains isolated from CSF, skin and blood of human patients and from ticks

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Background *Borrelia garinii* is mostly associated with central nervous system infections in Europe. Several molecular methods are available to characterise *Borrelia* strains; one of them, *Mlu*I-large restriction fragment pattern (LRFP), delineate *B. garinii* isolates into 7 subgroups, Mlg1 – Mlg7. The aim of the study was to assess discriminatory power of multilocus sequence typing (MLST) and to compare the findings obtained with *Mlu*I-LFRP and MLST for Slovenian *B. garinii* isolates.

Methods 44 *B. garinii* isolates, 34 obtained from patients with Lyme borreliosis (27 originating from cerebrospinal fluid, 5 from skin samples, 2 from blood samples) and 10 from ticks, were typed by *Mlu*I-LRFP and MLST. *Mlu*I-LRFP and MLST were performed as previously described by Ružić-Sabljić et al and Margos et al. New alleles and STs were added to the *Borrelia* MLST database (http://pubmlst.org/borrelia) hosted by University of Oxford, UK.

Results According to MluI-LRFP, the majority of isolates (40/44; 90.9 %) were identified as *B. garinii* Mlg2, 2/44 (4.5 %) as *B. garinii* Mlg4, and 1/44 (2.3 %) as *B. garinii* Mlg1 or *B. garinii* Mlg3, respectively. MLST analysis resulted in 12 distinct STs (82, 85, 86, 87, 93, 180, 244, 245, 246, 251, 574 and new ST); the most frequent ST was ST86 (15/44; 34.1%) encompassing isolates from CSF, ticks and blood. 11 human isolates, obtained mostly from CSF, were typed as ST 85 characteristic for *B. bavariensis*. Among *B. garinii* Mlg2 tick isolates, two gave mixed sequences with MLST typing, indicating mixed borrelia infections and in one new ST was determined.

Conclusions MLST has higher intraspecies discriminatory power in comparison to *Mlu*I-LRFP and is valuable for appraisal of the population structure of *Borrrelia*. Furthermore, it enables delineation of *B. bavariensis*. In Slovenia, *B. bavariensis* was established in 32.4 % of MluI-LRFP determined human *B. garinii* isolates.

P30 Published data do not support the notion that Borrelia valaisiana is human pathogenic

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The genospecies *B. valaisiana* was described in 1997 (Wang et al. 1997) and is found frequently in *Ixodes ricinus* ticks in Europe. It was noticed that more and more publications and reviews consider *Borrelia valaisiana* as human pathogenic. A Pubmed search was performed using the words "Borrelia valaisiana" and "human" to retrieve publications reporting on the presence of *B. valaisiana* in human samples. Such publications were screened and the evidence for human pathogenicity of *B. valaisiana* was scrutinized. Six publications from Europe and Asia reported the presence of *B. valaisiana* DNA but – to the best of our knowledge – no single cultured isolate from a human patient was reported. Several of the samples positive for *B. valaisiana* DNA were also positive for human pathogenic *Borrelia* genospecies such as *B. garinii, B. afzelii* or *B. burgdorferi* sensu stricto making it difficult to judge which of these was the symptom causing *Borrelia* species. In addition, data reported from Asia suggest misidentification of *B. valaisiana* instead of *B. yangtzensis*. The data provided and discussed in this poster, i.e. the scarcity of *B. valaisiana* DNA in human patient samples (n= 12 in 20 years), methodological ambiguities and the absence of a single cultured isolate combined with the relatively high prevalence of *B. valaisiana* in questing *I. ricinus* ticks, led us to propose that *B. valaisiana* is not human pathogenic.

Wang G, van Dam AP, Le Fleche A, Postic D, Peter O, Baranton G, de Boer R, Spanjaard L, Dankert J: Genetic and phenotypic analysis of *Borrelia valaisiana* sp. nov. (*Borrelia* genomic groups VS116 and M19). *Int J Syst Bacteriol* 1997, 47(4):926-932.

P31 In vitro susceptibility testing of antibiotics against nineteen Borrelia burgdorferi sensu lato strains from Republic of Serbia

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Previous genotyping of causative agents of Lyme borreliosis, *Borrelia burgdorferi* sensu lato, isolated from *Ixodes ricinus* ticks from Serbia showed high species and subspecies diversity.

The aim of the present study was to determine *in vitro* susceptibility of representative local strains (nine *B. afzelii*, three *B. garinii*, five *B. lusitaniae* and two *B. valaisiana*) to antibiotics, most frequently used for treatment of Lyme borreliosis (amoxicillin, ceftriaxone, cefuroxime, azithromycin, doxycycline) and amikacin.

The broth microdilution assay was used to investigate *in vitro* susceptibility. Borreliae were cultured in Barbour-Stoenner-Kelly-H (BSK-H) media and final inoculum was 10^5 *Borrelia* cells/mL. The microtitre plates were incubated at 33°C for 72 h under anaerobic conditions. After incubation, all wells were examined and compared to positive control using dark-field microscopy.

The MIC range for all *Borrelia* isolates was as follows: amoxicillin, 0.125-2 mg/L; ceftriaxone, 0.016-0.125 mg/L; cefuroxime, 0.063-1 mg/L; azithromycin, 0.0017-0.11 mg/L; doxycycline, 0.125-1 mg/L; amikacin, 32-512 mg/L. Obtained MICs indicated that all tested *Borrelia* isolates were susceptible to all tested antibiotics, except to amikacin.

There were small but statistically significant differences in MICs among different antibiotics against various *Borrelia* isolates ($P<0.05$). The MICs of amoxicillin and azithromycin appeared to be significantly lower in *B. lusitaniae* and *B. valaisiana* than in *B. afzelii* and *B. garinii* ($P<0.05$), respectively. The MICs of doxycycline in *B. garinii*, *B. lusitaniae* and *B. valaisiana* were lower than in *B. afzelii* ($P<0.05$). There were no statistically significant differences between the tested isolates with respect to their *in vitro* susceptibilities to ceftriaxone and cefuroxime, ($P>0.05$).

This study is the first report on *in vitro* susceptibility of local isolates from Serbia to antibiotics. The results indicate that antibiotics used in the treatment of Lyme borreliosis are effective against *Borrelia* isolated from ticks in our country.

P32 Epidemiology of Ixodes tick-borne borreliosis in Russia

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Ixodes tick-borne borreliosis **(**ITBB) is a notifiable disease in Russia. ITBB with erythema migrans (EM) is considered the “classical" Lyme borreliosis (LB). Historically, ITBB without EM - when antibody responses to *Borrelia burgdorferi* sensu lato are detected - was considered as LB without EM. However, recently we have shown that in most such cases DNA of the relapsing fever spirochete *B.miyamotoi*, but not *B.burgdorferi* sensu lato, is detected in patients' blood, as well as specific antibodies against GlpQ and variable major proteins (Vmps) of *B.miyamotoi.* In addition, in ITBB without EM patients with 'antibodies against *B.burgdorferi* sl' these antibody response appear not to be specific because of cross-reactivity of flagellin, and other antigens also present in *B. miyamotoi,* in commercial diagnostic assays for LB. The objectives of this study were 1) to estimate the incidence and other epidemiological features of ITBB without EM in Russia; 2) to confirm that most cases of ITBB without EM are actually *B.miyamotoi* disease (BMD).

We have collected and are analyzing information from various regions across Russia, namely Udmurt Republic, Bashkir Republic, Sverdlovsk, Omsk, Altai, Vologda, and Kaluga Provinces and according to long-term registration 10-40% of all ITBB cases were marked as “ITBB without EM" cases. When “ITBB without EM" cases were studied by qPCR and/or serological methods, approximately 50% and more was diagnosed as BMD. For example, in Sverdlovsk Provinces between 2009 and 2014, 5165 ITBB cases - including 1999 “ITBB without EM" cases - were registered. This corresponds to an annual incidence of “ITBB without EM" of about 7.7 cases per 100,000 population. Averaging the results from different provinces and extrapolating these to the whole country, we expect at least 2000 clinical BMD in Russia every year.

This study was supported by the grant of Russian Scientific Foundation (project 15-15-00072).

P33 Prevalence of Borrelia infections in the United Kingdom and the development of an enhanced testing regime

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The Rare and Imported Pathogens Laboratory (RIPL) at Public Health England (PHE), Porton Down provides a reference service for the laboratory diagnosis of *Borrelia* infections in the United Kingdom. The assays in use at RIPL currently focus on the diagnosis of Lyme Borreliosis with limited capability of diagnosing infections with the Relapsing Fever clade of *Borrelia*. The specificity of the assays in use to be able to detect infection with UK circulating strains of *Borrelia* has also not been ascertained.

One area of interest is to determine the prevalence of Relapsing Fever in locally acquired infections as well as infections in returning travellers. Recombinant Glycerophosphodiester Phosphodiesterase (GlpQ) protein specific to the Relapsing Fever clade of *Borrelia* will be used to develop an ELISA assay which has been previously demonstrated by TG Schwan et al. (1996). A panel of samples from the RIPL sample archive will then be tested using the GlpQ ELISA to determine seroprevalence in UK endemic cases and cases in returning travellers. After initial identification of antibodies, further work will look at other variable membrane proteins to determine immunodominance and their potential benefit for early/late diagnosis of Relapsing Fever.

Another area of interest is the suitability of antigens used in commercially available assays to detect antibodies raised during infection with UK *Borrelia* genospecies. This will be done using a proteomic and genomic approach in order to analyse commercially available antigens and compare the protein sequences with that of local Borrelia to determine differences in immunoreactivity. A strain collection of different *Borrelia* genospecies will also be created with input from collaborators within PHE and the NHS in order to provide valuable sequence data for these projects as well as any future research.

P34 How much Lyme borreliosis is actually seen in primary health care in Scotland?

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Lyme borreliosis is not a notifiable disease in Scotland.Current incidence data is dependent on the reporting of new seropositive patients by the National Lyme Borreliosis Testing Laboratory, Inverness. It is recommended that general practitioners (GPs) presented with patients exhibiting the classic erythema migrans rash after tick bite/exposure should treat empirically without testing. However, this means that current figures are an underestimate of what is actually happening in Scotland.This project aimed to identify the proportion of patients diagnosed empirically at GP practices in Highland region, an endemic area of Lyme borreliosis, and compare it to the laboratory data for those practices.

Initially 20 GP practices were identified to be part of the study but this was reduced to three practices after a pilot study indicated that the time taken to extract the data from the practices was enormous (2 years for three practices, 0.5 WTE research nurse).Therefore, three large practices (Nairn, Culloden and Fort William) were analysed by identifying cases from the READ codes (GP database coding) and free text indicating treated, suspected or confirmed Lyme borreliosis.Patients coded for 'Lyme disease', 'erythema migrans' or 'rash' including a free text entry indicating 'Lyme' for which antibiotics were prescribed were also included.These figures were compared to the laboratory data for the years 2010-2012.

The ratio of laboratory cases: GP cases was 3.8 (Fort William), 3.8 (Culloden) and 3.9 (Nairn) with an average of 3.83.Assuming this ratio for laboratory-based incidence figures for the Highland region the incidence would increase from 44.1/100 000 to 168.9/100 000.These data indicate that Lyme borreliosis is of greater public health significance than previously thought.The study has also demonstrated that data mining is difficult within the primary care setting and in future should be performed using a specifically developed software program.

P35 The temporal and spatial dynamics of tick-borne disease in Cumbria

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Borrelia burgdorferi sensu lato (s.l.) group of spirochetes are the causative agents of Lyme borreliosis, of which an estimated 3000 cases occur annually in the UK. Another group of spirochetes known as relapsing fever- like Borrelia can cause disease in humans. Recently *Borrelia miyamotoi,* a relapsing fever- like borrelia, has been reported in the south of England and in Scotland. *Ixodes ricinus* is the principal vector of both of these spirochetes in the UK, and this tick species is widely distributed across the country.

The aims of this project were to investigate the temporal and spatial dynamics of *B. burgdorferi* s.l. populations and to test for the presence of *B. miyamotoi* at three sites in southern Cumbria.

Questing *I. ricinus* ticks were collected between June 2013 and November 2016 and tested for the presence of *B. burgdorferi* s.l. and *B. miyamotoi* usingrealtime PCR and conventional PCR. The prevalence of *B. burgdorferi* s.l. infections in ticks at each site ranged from 0.5% to 15.8%. Four genospecies were detected across the three sites although the contribution that each genospecies made to the borrelial population at each site varied markedly. A temporal change was observed in the overall prevalence of *B. burgdorferi* s.l. at each site. *B. miyamotoi* was detected at one of the three sites.

B. burgdorferi s.l. infection prevalence in ticks varies spatially and temporally thus reliance on single cross-sectional surveys to estimate local Lyme borreliosis risk could be misleading. More work is needed to understand the ecological determinants of the observed variation.

P36 Structural insights into tick attachment cement

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In many cases ixodid ticks do not solely rely on the mouthparts for a firm attachment to their hosts, but additionally secrete cement from their salivary glands. Cement acts as adhesive and sealant during the long feeding periods. Due to its natural functions, cement is of interest for the research on alternatives to established medical adhesives. The aim of the current study is to gain more information on the structure and composition of the adhesive material.

Amblyomma hebraeum and *Dermacentor marginatus* ticks were fed in artificial feeding units with silicone membranes replacing the host skin. Cement was harvested and analysed by histochemistry, biochemistry and Electron Dispersive X-Ray Analyses (EDX). Histochemical staining included Biebrich Scarlet, Periodic acid-Schiff, Martius Scarlet Blue and Alcian blue. Cement was further analysed by gas chromatography electron ionization tandem mass spectrometry after acidic hydrolysis in order to identify the amino acid composition.

Even though the cement appears homogenous macroscopically, there is an internal compartmentation characterized by different densities and composition. Histochemical staining indicated basic proteins as important component of cement, but there are also some carbohydrates present. EDX primarily revealed elements like carbon and nitrogen. The most abundant amino acid found was glycine.

The different densities and internal compartmentation of cement probably reflects changes of its composition during the secretory process. High amounts of glycine are in agreement with cement of other tick species and some other animal adhesives. Further biochemical analyses will bring more detailed information on the composition of cement including the protein contents and the nature of the carbohydrate. This work is funded by the FWF grant # P 28962 and supported by the European Cooperation in Science and Technology (COST Action TD0906).

P37 Amblyomma hebraeum: analysis of tick attachment cement

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Tick attachment cement, produced by the salivary glands of ixodid ticks prior and during their blood meal, is a proteinaceous substance that rapidly hardens when secreted. It is suggested that the main function of this adhesive is to strengthen the attachment by the ticks' mouthparts to the vertebrate host. Until now the cement and especially its adhesive properties are still largely unexplored and therefore it is an interesting and promising field of research. For the investigation of the cement and its biochemical composition, GC-EI-MS/MS (gas chromatography electron ionization tandem mass spectrometry) and one dimensional SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) were performed. Cement was collected from *in vitro* fed *Amblyomma hebraeum* (Ixodidae) using an artificial membrane feeding system. This system allows an easy and contamination-free collection of tick cement cones.

After hydrolyzation and derivatization of tick cement samples, GC-EI-MS/MS allowed to get an insight into the amino acid composition of the adhesive. It was found that the cement mainly contains non-polar amino acids with glycine as the major component. This is in good agreement with literature, which lists amino acid compositions from genomic data.

Proteins were solubilized using different buffer systems and protein concentration was determined with protein assays. SDS-PAGE allowed to resolve various proteins between 20 and 80 kDa, which were not investigated further so far. However, compared to literature this molecular weight range contains the main tick proteins present in the cement and therefore seems to be very interesting especially for the adhesive properties.

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P38 LymeProspect: ongoing prospective study into long-term effects of Lyme borreliosis and determinants for persisting symptoms in the Netherlands

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Introduction Approximately 5-20% of patients treated for Lyme borreliosis report disabling persisting symptoms. The aim of the LymeProspect study is to prospectively determine the incidence and prevalence of persisting symptoms in patients with confirmed Lyme borreliosis after antibiotic treatment. Moreover, we aim to assess microbiological, immunological, genetic, clinical, cognitive-behavioral, and epidemiological determinants for development of these symptoms. This study started in 2015 and continues through 2019. From 2017 on, children are also included into the study.

Methods We strive to include 2000 adult patients and 300 children with confirmed erythema migrans (EM) or disseminated Lyme manifestations at the initiation of antibiotic treatment. During one year follow-up, participants answer online questionnaires and provide blood samples. Subsets of patients undergo skin biopsies. We measure occurrence and severity of persisting symptoms with questionnaires based on Dutch population norm scores. Potential determinants of persisting symptoms are assessed by serology, cellular immune responses, PCR for *Borrelia* and other tick-borne pathogens, gene expression arrays, antibiotic plasma levels, and validated questionnaires on symptoms (CIS, CFQ, SF-36-pain, PHQ-15, and specific questionnaires for children), disabilities, comorbidity and cognitive-behavioral variables.

Preliminary results Between 2015 and early 2017, 650 adult patients with Lyme borreliosis have been included. The majority (94%) of participants had an erythema migrans, whereas acrodermatitis chronica atrophicans is the most common disseminated manifestation in our cohort (3.5%). Analysis of the immunological responses, measured by whole blood and PBMC stimulations, proved to be highly variable and results will be linked to clinical and genetic data in the near future. We are currently analyzing the prevalence of persisting symptoms. Moreover, serological results of our patients before and after treatment will be presented at this symposium.

Conclusion The ongoing LymeProspect study will provide important insights into long-term effects of Lyme borreliosis and determinants for persisting symptoms in both adults and children.

P39 LymeMAP: a European collaborative project to investigate the feasibility of producing a real-time, interactive app containing tick maps and current information on Lyme borreliosis

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The LymeMAP project involves the integration of epidemiological data and European Space Agency space assets to reduce the incidence and morbidity of Lyme borreliosis in the Highlands of Scotland. Its aim was to provide a "real-time" interactive, spatially accurate identification and risk management tool (app) that will respond to the needs of health care professionals, resident populations and tourists.

Retrospective epidemiological and patient demographic data from the Scottish National Lyme borreliosis testing laboratory, Raigmore hospital and an NHS Primary Care project was integrated with European Space Agency data. Earth Observation data e.g. precipitation, actual and relative evapotranspiration, day and night-time land surface temperature, enhanced vegetation index and land use and Global Navigation Satellite System data were used to geo-localise *in situ* data eg tick collections or location of disease cases to identify Lyme borreliosis "hot spots" and influential factors. Stakeholders and end-users were engaged to identify clinical interests and preferred knowledge exchange mechanisms and results integrated with community engagement work undertaken by the Centre for Rural Health, University of the Highland and Islands, Inverness. The use of Satcom capabilities to input data from public and other users to website portals was investigated and optimal spatio-temporal models for the identification of trends across space and time along with other risk factors were created.

The results of the collaborative project indicated that it was feasible to create an app to improve awareness of Lyme borreliosis among the general public, healthcare and commercial communities in the Highlands and create real-time maps of tick abundance using existing epidemiological and space technology data and data uploaded to the app from the general public. The collaborative group is approaching ESA for funding for the development stage of the project.

P40 Course and outcome of erythema migrans in pregnant women

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Background Information on Lyme borreliosis (LB) during pregnancy is limited.

Methods The course and outcome of erythema migrans (EM) in 304 pregnant women was assessed and compared with that in age-matched non-pregnant women with EM. The frequency of unfavorable outcome of pregnancies was also evaluated.

Results The pregnant women reported constitutional symptoms less frequently than the non-pregnant women (22.4% vs 37.2%, $P<0.001$). Pregnant women diagnosed with EM later during pregnancy had a lower probability of reporting constitutional symptoms (OR=0.97 for 1-week difference in gestation week at diagnosis of EM, 95% CI: 0.94–0.99, $P=0.02$).

The outcome of pregnancy was unfavorable in 42/304 (13.8%) patients: preterm birth in 22/42 (52.4%), fetal/perinatal death in 10/42 (23.8%), and/or anomalies in 15/42 (35.7%). Higher risk of unfavorable outcome was associated with an earlier gestation week at diagnosis of EM, and with older age of patients, shorter duration of EM before diagnosis, and the presence of multiple EM or borreliae isolated from blood. However, only the gestation week at EM diagnosis was significantly associated with a higher risk of unfavorable pregnancy outcome, and several patients had potential explanation(s) other than LB for unfavorable outcome.

Conclusions The course of early LB during pregnancy is milder than in age-matched non-pregnant women. The outcome of pregnancy with the treatment approach used in the present study (i.v. ceftriaxone 2 g once daily for 14 days) is favorable. Multivariable analyses showed that patients who develop EM in the early stages of pregnancy might have a higher risk of an unfavorable outcome.

P41 Erythema migrans: course and outcome in patients treated with rituximab

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Objectives To assess the course and outcome of erythema migrans (EM) in adult patients treated for underlying disease with rituximab, the anti-CD20 monoclonal antibody that influence immune system B cells.

Methods Information obtained from database on adult patients diagnosed with EM at our institution from 2008–2016, revealed 5 patients with typical EM, 3 females and 2 males, aged 65 (55–66) years, who were receiving rituximab for rheumatoid arthritis (2 patients), hematological malignancies (1 had Waldenström's macroglobulinemia, 1 follicular lymphoma) oranti-MAG peripheral neuropathy (1 patient). In addition to rituximab, 3 of them were receiving corticosteroids, and 2 methrotrexate or bortezomid, respectively).

Results The main clinical findings were as follows: 3 patients recalled tick bite, incubation period was 27 (14–31) days, interval to diagnosis 7 (4–68) days; 3/5 (60%) patients presented with multiple EM – they had median 12 (3–16) skin lesions; only one patients had constitutional symptoms. Borrelial serum antibodies were present in 2/5 (40%) patients. Borreliae were isolated from skin in 4/4 (100%) patients (1 isolate was *B. burgdorferi* s.s., 3 *B. afzelii*) and from blood in 2/4 (50%) patients (1 *B. burgdorferi* s.s., 1 *B. afzelii*). Patients with multiple EM were treated with iv ceftriaxone, while patients with solitary EM received either oral azithromycin or cefuroxime axetil. Skin lesions disappeared 5 (4–28) days after the onset of antibiotic treatment. Clinical course during one-year follow-up was smooth; no treatment failure was registered.

Conclusions In patients receiving rituximab due to underlying illness, signs of disseminated Lyme borreliosis (60%) and isolation rate of borreliae from blood before antibiotic treatment (50%) were unusually high in comparison with the corresponding findings in immunocompetent adult population with EM (3–8% and about 1%, respectively). However, the outcome of early Lyme borreliosis one year after antibiotic treatment was excellent.

P42 Comparison of antibody response to immunodominant borrelial antigens in pediatric Lyme arthritis

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Purpose Laboratory diagnosis of Lyme arthritis is based on the detection of a high level of immunoglobulin G (IgG) antibodies against Borrelia burgdorferi sensu lato. Whole-cell antigens contain epitopes which may cross-react with epitopes of other bacterial species. The reactivity to each of the recombinant proteins as antigens was examined in order to evaluate their diagnostic value in pediatric Lyme arthritis (LA).
Patients and Methods In total, 204 samples obtained from 69 children were evaluated in the first panel. Arthritis (minimum one-time or repeated swelling of large joints) was the presenting feature in all of them. All children were treated by one course of antibiotics. The second panel consisted of 34 samples from controls (23 children with rheumatoid diseases and 11 samples from healthy children). The tested immunoblot (BlueBLOT-LINE Borrelia IgG, CZ) is based on highly specific recombinant Borrelia antigens known to be proteins with high immunoreactivity in Lyme disease patients. Reactivity of these antigens was compared to the recombinant EIA test based on the selected antigen fragments p17, OspC, p39, p41i, p83 and VlsE for IgG.
Results The reactivity of both methods EIA (87.3%) and BLOT (90.2%) in the group of LA patients was similar. Most of the samples were positive in seven antigens, four being of B. burgdorferi sensu stricto origin, VlsE, p41, p39 and OspA; two being of B. garinii origin, VlsE and p58; and p83 of B. afzelii. P values were between 0 and 0.017 (Generalized Estimating Equations Model). One sample was positive in EIA test and three samples were positive in BLOT test in the group of children with rheumatoid arthritis. Cohen's kappa coefficient is 0.476 for BLOT test in the second panel.
Conclusion High confidence level of reactive antigens correlates very well with their species origin and corresponds with their association to Lyme arthritis.

P43 Asymptomatic Lyme borreliosis is common in Sweden and may be distinguished from Lyme neuroborreliosis by sex, age and specific immune marker patterns

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Background Determinants of an asymptomatic course of Lyme borreliosis (LB) remain largely unknown. The aim of this study was to assess the extent, sex and age profiles of asymptomatic *Borrelia* infection in a LB endemic area in Sweden and to map blood cellular *Borrelia*-specific immune marker responses in individuals with a previous asymptomatic LB course compared with patients previously diagnosed with Lyme neuroborreliosis (LNB).

Methods A large group of 1113 healthy blood donors was screened for multiple IgG anti-*Borrelia* antibodies and asked to complete a health inquiry regarding previous LB. A group of subjects with anti-*Borrelia*-specific IgG antibodies but no previous history of LB (asymptomatic LB, n=60) was identified together with 22 cases of previous LNB. Whole *Borrelia* spirochetes, strains *B. afzelii* ACA1 and *B. garinii* Ip90, were used for whole blood stimulations, whereas outer surface protein-enriched fractions of the same strains were used for ex vivo stimulation of peripheral blood mononuclear cells (PBMCs). An extensive panel of immune markers was analysed in the supernatants after stimulation using multiplex bead arrays, and *Borrelia*-specific secretion was determined by subtracting the spontaneous secretion.

Results A total of 125/1113 blood donors reported previous clinical LB. In contrast, 66 donors denied previous LB but showed multiple IgG anti-*Borrelia* antibodies; these were defined as asymptomatic subjects, of whom 60 were available for further studies. The asymptomatic subjects consisted of significantly more men and had a younger age compared with the LNB patients ($p \leq 0.01$). Discriminant analysis revealed a distinct pattern of sex, age and PBMC *B. garinii*-specific levels of IL-10, IL-17A and CCL20 discriminating asymptomatic subjects from LNB patients.

Conclusions This study confirms that asymptomatic *Borrelia* infection is common in Sweden. The findings further suggest that male sex, younger age, and *Borrelia* subspecies together with specific immune responses may be associated with an asymptomatic course.

P44 Neuroborreliosis - 23 years of experience

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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.

Currently in Europe two step diagnostic approach involving ELISA test and Western Blot test is characterised by high specificity and sensitivity. This diagnostic tool has been available in Poland for several years already. After introduction ofintrathecal synthesis 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 hospitalised between 1993 - 2008 whereas the second group was hospitalised between 2009 -2015. In the latter oneintrathecal synthesis of anti-Borrelia burgdorferi antibodies were assessed. Introduction of the novel diagnostic tool enabled proper selection of patients with confirmed neuroborreliosis out of patients with suspected Lyme disease. Before 2008 hospitalisation rate due to LNB had been gradually increasing. Once the novel diagnostic tool has been introduced the hospitalisation rate decreased and is nowadays at a constant level. Patients hospitalised between 2009-2015 presented more frequently with neurological symptoms such as Bannwarth's triad (three-fold higher incidence), concomitant meningitis with facial nerve paralysis (five-fold higher incidence), meningitis (two-fold higher incidence) and other cranial neuropathies. Owing to new diagnostic methods proper differentiation of neuroborreliosis from diseases such as multiple sclerosis and Parkinson's disease is possible. Consequently, the incidence of neurological symptoms similar to MS in the group treated between 2009 and 2015 had decreased

P45 Clinical and imaging findings in patients with Lyme neuroborreliosis

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Introduction Lyme borreliosis is a multisystem infection caused by the tick-transmitted spirochete, *Borrelia burgdorferi sensu lato*. Nervous system invasion occurs in 10-15% patients with Lyme borreliosis, and can occur at any stage of the disease. This diagnosis of Lyme neuroborreliosis is based on clinical symptoms and findings in the cerebrospinal fluid but is not always conclusive. The present study was contucted to focus on the clinical and the imaging findings of the LNB.

Materials and methods A retrospective study of the medical records of all patients diagnosed with LNB and those who were evaluated with magnetic resonance imaging (MRI) between years 2015-2017 were carried out.All the patients satisfied the European Federation of Neurological Societies criteria for definite LNB.

Results The patients' age ranged from 19 to 79 years, with a median age of 49. Among 21 patients with proved LNB and in whom MRI was performed, 8 showed abnormalities on MRI. Five of them had multifocal brain lesions diagnosed at imaging (including rarely seen inflammatory changes in the brainstem), 2 patients demonstrated contrast enhancement of cranial nerves and 1 had hydrocephalus. The remaining patients had normal structural MRI.

Conclusion LNB is easily treatable with appropriate antibiotics, but establishing the diagnosis is often difficult. Brain MRI are usually normal in most patients with LNB. MRI findings in LNB are usually not specific. Sometimes, MRI as a noninvasive imaging tool revealing signal alterations in the brainstem and contrast enhancement of cranial nerves may help in making the diagnosis.

P46 Occurrence of erythema migrans in children with Lyme neuroborreliosis and association to outcome

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Erythema migrans (EM) is the most common manifestation of Lyme borreliosis (LB), caused by the spirochete *Borrelia burgdorferi senso lato*. The infection can disseminate into the nervous system and cause Lyme neuroborreliosis (LNB), the second most frequent LB manifestation in children. The aim of this study is to describe the occurrence of EM among children with LNB and to describe possible differences in clinical characteristics or outcome between LNB patients with and without EM.

Material and methods Children being evaluated for LNB in central Sweden during 2011-2015 underwent a clinical examination, laboratory testing and filled out a questionnaire about duration and nature of symptoms, EM and the child's health. Children were categorized as LNB according to European guidelines.

Results The occurrence of EM among children with LNB was 37 out of 103 (36%). Sex, age, known tick bite, clinical features, duration of neurological symptoms or clinical outcome did not differ significantly between LNB patients with or without EM. Facial nerve palsy was significantly more common among children with EM in the head and neck area.

Conclusion EM occurred in 36% of children with LNB and the location on the head and neck was more common among children with facial nerve palsy. EM was not associated to clinical characteristics or outcome. Thus, the occurance of EM in children with LNB cannot be useful as a prognostic factor for clinical outcome. This aspect has not previously been highlighted, but seems to be relevant for the pediatrician in a clinical setting.

P47 Characteristics of patients with peripheral facial palsy due to Borrelia burgdorferi sensu lato infection

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Objectives The aim of our study was to compare clinical and laboratory characteristics of patients with peripheral facial palsy (PFP) due to borrelia infection with those obtained in patients with PFP of unknown aetiology.

Patients and methods Adult patients, who presented to our department from January 2006 to December 2013, with PFP, had undergone lumbar puncture and were tested for the presence of borrelial IgM and IgG antibodies in serum and CSF with an indirect chemiluminescence immunoassay, qualified for the study. Patients with PFP who had obvious signs/symptoms indicating a disease other than Lyme borreliosis (LB), were excluded.

According to the clinical and microbiological criteria patients with PFP were classified into three groups: those having confirmed LB, those with possible LB, and patients with PFP of unknown etiology.

Results Of 589 patients diagnosed with PFP during the eight-year period, 436 patients (240 males and 196 females) with the median age of 42.5 years (15–87 years), qualified for the study. Among them 64 (14.7%) patients fulfilled the criteria for confirmed LB, in 120 (27.5%) patients the diagnosis of possible LB was established, and in 252 (57.6%) patients the cause of PFP remained unknown.

In comparison to patients with the unknown cause of PFP patients with confirmed LB were older, more often reported tick bites, had more frequently LB in the past, more often complained of constitutional symptoms and radicular pain, and more often had bilateral palsy and CSF pleocytosis. There were no differences in regard to the frequency of constitutional symptoms, radicular pain, bilateral palsy or CSF pleocytosis in the group of patients with possible LB and patients with the unknown cause of PFP.

Conclusions Tick bites, constitutional symptoms and radicular pain, as well bilateral palsy and CSF pleocytosis strongly point to borrelial etiology of PFP.

P48 Depressive symptoms in patients referred to a tertiary Lyme center: high prevalence in those without evidence of Lyme borreliosis

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Background Controversy exists whether mood disorders, such as depression, are associated with Lyme borreliosis. The study objective was to assess prevalence of moderate/severe depressive symptoms in subgroups of patients referred to a tertiary Lyme center in order to investigate whether depressive symptoms can be used in clinical practice to discriminate for Lyme borreliosis.

Methods This retrospective cohort study included adult patients who visited a tertiary Lyme center between January 2008 and December 2014. Prior to medical consultation serum samples were taken and the Beck Depression Inventory II was completed to assess depressive symptoms. Lyme diagnosis was retrospectively extracted from the patient's medical record. Patients were classified into categories: 1) no clinical Lyme borreliosis and negative serology; 2) no clinical Lyme borreliosis and positive serology; 3) clinical Lyme borreliosis and negative serology; 4) clinical Lyme borreliosis and positive serology. Prevalence of moderate/severe depressive symptoms was calculated. Using logistic regression ORs with 95% CI were calculated for moderate/severe depressive symptoms.

Results In total, 1454 patients were included. Prevalence of moderate/severe depressive symptoms was lowest in patients with no clinical Lyme borreliosis and positive serology (15.3%), higher in patients with clinical Lyme borreliosis with positive and negative serology (19.3% and 20.9% respectively), and highest in patients with no clinical Lyme borreliosis and negative serology (29.3%). The adjusted OR for moderate/severe depressive symptoms in patients with Lyme borreliosis and positive serology was 0.71 (95% CI 0.50-1.03) compared to patients with no Lyme borreliosis and negative serology.

Conclusions The prevalence of depressive symptoms was similar in patients with Lyme borreliosis compared to patients with no evidence of infection. This suggests depressive symptoms cannot be used to discriminate for Lyme borreliosis in a tertiary Lyme center.

P49 Children suspected of Lyme borreliosis referred to a tertiary Lyme center: prevalence of severe fatigue and depressive symptoms

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Background It is unclear whether severe fatigue or depressive symptoms are associated with Lyme borreliosis (LB) in children. The study objective was to assess the prevalence of severe fatigue and depressive symptoms in children with and without LB referred to a tertiary Lyme center.

Methods A retrospective cohort study was performed including all children aged <18 years old who visited the tertiary Lyme center between January 2008 and December 2014. Before medical consultation serum samples were taken and questionnaires were completed to assess severe fatigue and depressive symptoms. Lyme diagnosis was retrospectively extracted from the patient's medical record. Patients were classified based on clinical findings and serology results. Prevalence of severe fatigue and depressive symptoms was calculated.

Results Eighty children were included of which 32 (40%) had no clinical LB and negative serology, 19 (24%) had no clinical LB and positive serology, 17 (21%) had clinical LB and negative serology, and 10 (13%) had clinical LB and positive serology. All 27 children with clinical LB experienced an infection in the past. Overall prevalence of severe fatigue was 53%. This prevalence was not significantly different in the four patient groups (P=0.496), and ranged from 41% in children with clinical LB and negative serology to 63% in children with no clinical LB and negative serology. Overall prevalence of depressive symptoms in children aged >13 years old was 33%. Among the four patient groups, the prevalence of depressive symptoms was not significantly different (P=0.476)

Conclusion Prevalence of severe fatigue and depressive symptoms in children referred to a tertiary Lyme center was high. The prevalence in children with previous LB did not exceed the prevalence in children without clinical LB. Regular screening for fatigue and depressive symptoms may facilitate timely referral for psychological diagnosis and treatment.

P50 Borrelia burgdorferi sensu lato in cerebrospinal fluid – can the sensitivity of molecular diagnostics in neuroborreliosis be improved?

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Lyme neuroborreliosis (LNB) is the second most common manifestation of *Borrelia burgdorferi* sensu lato (s.l.) in Europe. Laboratory diagnosis of LNB is mainly based on detection of specific intrathecal antibodies. However, the sensitivity of antibody detection is limited in early cases due to the natural delay of the antibody response. Therefore, direct detection of *B. burgdorferi* s.l. by PCR may be useful as a supplementary tool in early LNB. Although PCR is not suitable as a primary diagnostic tool, it may serve as a supplementary method to serology for certain conditions, but also in confirmation and genotyping of the infecting *Borrelia* spirochetes. In previous studies, the sensitivity of *Borrelia*-specific PCR in blood and cerebrospinal fluid (CSF) has in general been low (10-30%), which may be a result of the low number of spirochetes in these samples. Different centrifugation time and speed, sample volumes and storage conditions have been used and may have contributed to the discordant sensitivity and specificity. However, to our knowledge, no systematic evaluation of the pre-analytical conditions has been performed and no general pre-analytical recommendations have been published. The aim of the study is to establish recommendations for detection of *Borrelia* in CSF by optimization of pre-analytical steps, such as centrifugation time, centrifugation speed, sample volume and storage conditions. For this purpose CSF spiked with cultured *B. burgdorferi* s.l. spirochetes will be used. The optimal pre-analytical procedure will then be applied on an existing set of patient samples and the PCR results will be compared with serological results on the same patients. By establishing pre-analytical recommendations we hope to increase the sensitivity of the molecular methods. The project is expected to have direct implication for both clinical and laboratory routine.

P51 Immunological biomarkers in the cerebrospinal fluid and the association to clinical presentation and recovery in children with Lyme neuroborreliosis

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Background Lyme neuroborreliosis (LNB) is caused by *Borrelia (B) Burgdorferi*, the most common tick-borne zoonosis in the Northern hemisphere. Pediatric patients often present with short duration of symtoms and laboratory testing cannot always confirm the LNB diagnosis. Consequently, better diagnostic markers are needed. Previous studies have been looking for new markers in the cascade of cytokines and chemokines and the CXCL13 has been shown to be a sensitive biomarker. In this study we explore a panel with 41 cyto/chemokines in a large pediatric population.

Aim To find early biomarker for LNB, but also to investigate if any cyto/chemokine were associated with clincial presentation, sex, age, duration of neurological symptoms or recovery in children with LNB.

Methods Cerebrospinal fluid (CSF) from children being evaluated for LNB during 2011-2014 in central Sweden were collected (n=87). Patients were classified according to European guidelines into 3 groups: definite LNB, possible LNB and non-LNB. A control group of children with other neurological diagnoses was included (n=13). The CSF samples were analyzed with a Luminex based multiplex bead assay for 41 cyto/chemokines.

Results Most of the cyto/chemokines (except TGF-α, Flt-3L and IL-9) were elevated in LNB patients as compared to non-LNB and controls. The groups definite LNB and possible LNB differed significantly in a large number of cyto/chemokines and IL-13 and IFN-γ were elevated in LNB patients with short duration of neurological symptoms. No difference in cyto/chemokines could be seen in relation to recovery.

Conclusion: The majority of the cyto/chemokines could potentially be useful as biomarkers for LNB in the future. The cytokines IL-13 and IFN-γ could possibly be suitable as early markers for LNB in children since they are elevated in patients with short duration of neurological symptoms. No cyto/chemokine were found to be a reliable prognostic marker for clinical recovery

P52 The recomBead Borrelia antibody index, CXCL13 and total IgM index for laboratory diagnosis of Lyme neuroborreliosis in children

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Background For laboratory diagnostics of Lyme neuroborreliosis the recomBead Borrelia antibody index (AI) assay has shown promising results in a mixed age population, but has not previously been evaluated with specific focus on paediatric patients. The aim of the study was to evaluate the recomBead Borrelia AI assay in cerebrospinal fluid (CSF) for laboratory diagnosis of Lyme neuroborreliosis in children. We also wanted to explore whether early markers, such as CXCL13 and/or total IgM index in CSF could be useful as complementary diagnostic tools for paediatric patients with Lyme neuroborreliosis.

Methods Children being evaluated for Lyme neuroborreliosis in a Swedish Lyme endemic area, were included in the study (n=146). Serum and CSF were collected on admission. Patients with other specific diagnoses were controls (n=15). The recomBead Borrelia AI assay and the recomBead CXCL13 assay (Mikrogen) were applied together with total IgM index.

Results The overall sensitivity for recomBead Borrelia AI (IgM and IgG together) was 74% and the specificity was 97%. However, the highest sensitivity (91%) at an acceptable level of specificity (90%) was obtained by a combination of all three tests (recomBead Borrelia AI, CXCL13 and total IgM index) with a positive predictive value of 84% and a negative predictive value of 95%.

Conclusion The recomBead Borrelia AI assay performs quite well in laboratory diagnosis of Lyme neuroborreliosis in children. It may be used in combination with early markers, such as CXCL13 and total IgM index, in order to increase the sensitivity in paediatric patients with short duration of symptoms.

P53 CXCL13 detected in CSF of patients suffering from tick-borne encephalitis, aseptic meningitis, Lyme neuroborreliosis and etiologically not identified neurologic disorder

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The aims of the study were to determine and compare the concentration of chemokine CXCL13 in cerebrospinal fluid (CSF) of patients with Lyme neuroborreliosis (LNB) and various other neurological conditions applying a Luminex based assay and ELISA, and to find factors associated with CXCL13 concentration.
CSF samples obtained from four clinically well-defined groups of patients were chosen for the analyses. The groups consisted of 25 patients with established LNB, 25 patients with suspected LNB, 25 patients with tick-borne encephalitis (TBE), and 25 patients with aseptic meningitis/meningoencephalitis other than TBE.

The performance of the Luminex recomBead CXCL13 assay (Microgen, Neuried, Germany) and ELISA (Euroimmun, Lübeck, Germany) was assessed by receiver operating characteristics. Demographic variables, CSF findings, and history of erythema migrans were assessed as possible predictors for CXCL13 CSF concentrations by a general linear model.

RecomBead showed a sensitivity of 88% (68.8-97.5%) and a specificity of 96% (88.8-99.2%). For the ELISA the corresponding values were 96% (79.6-99.9%) and 86.7% (76.8-93.4%). The cutoff values determined by the maximum of the Youden index were >131 pg/mL for recomBead and >69 pg/mL for the ELISA. The CXCL13 concentration positively correlated with CSF lymphocyte count and positive borrelia-specific intrathecal antibody index ($p<0.05$).

CXCL13 is supportive for the diagnosis of LNB. The cutoff values depend on the tests used. However, the chemokine is not specific for a *Borrelia* infection and should not replace the well-established criteria for the diagnosis of LNB.

P54 Retrospective evaluation of various serological assays for the detection of neuroborreliosis

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Introduction The diagnosis of Lyme neuroborreliosis (LNB) consists of clinical symptoms as well as laboratory findings including cerebrospinal fluid (CSF) pleocytosis and evidence of intrathecal antibody production. Many commercial assays are available for the diagnosis of LNB, but the literature lacks studies on the evaluation of these assays on well-defined patient populations.

Methods We evaluated seven different assays for the detection of *Borrelia*-specific antibodies in serum and CSF. The assays used were: the C6 ELISA, the recomLine immunoblot, the IDEIA, the MEDAC, the recomBead, the Enzygnost II, and the SERION ELISA. We retrospectively performed these assays on all consecutive patients for which CSF&blood sample pairs (sampling time ≤24 hours) were sent to the laboratory between 2013-2016. Patients were divided in six groups based on the likelihood of having LNB or another disease, independent of the intrathecal production of *Borrelia*-specific antibodies.

Results The CSF&blood samples of 151 patients were tested; 5/151 (3.3%) were classified as definite LNB, 5/151 (3.3%) as probable LNB, 31/151 (20.5%) as possible LNB, 17/151 (11.3%) as non-*Borrelia* infectious CSF disease, 49/151 (32.5%) as non-infectious CSF disease, and 44/151 (29.1%) as unknown CSF disease. So far, we analysed the data of five tests, but data of all tests will be presented at the meeting. The sensitivity of the C6 ELISA was 100%; however, this test does not correct for a disturbed function of the blood-CSF barrier or a traumatic lumbar puncture. Sensitivity decreased in following order: Medac IgG (80%), recombead IgG and two-tier C6 ELISA/IgG immunoblot analysis (60%, each), and IDEIA IgG (40%). IgM analysis did not result in additional positive cases. The specificity of the tests was good (94%-100%).

Conclusion The results of the five assays differ substantially. If laboratories use the low sensitive assays, then this severe form of Lyme borreliosis may remain unrecognized.

P55 Borrelia bacteriophages for diagnosis and treatment of Lyme Disease and Relapsing Fever

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Bacteriophages (phages) are viruses that infect bacteria. They have been investigated for diagnosis and treatment of many types of bacterial infections. However, a very few studies have been carried out on phages that infect *Borrelia*, a group of Spirochaetal bacteria that are the causative agents of Lyme Disease (LD) and Relapsing Fever (RF). The disease is transmitted to humans through the bite of infected ticks and louses. Up-to-date, the diagnosis and treatment options for LD and RF are problematic. In this project, we aim to carry out a systematic approach to isolate and characterise *Borrelia* phages to obtain a better understanding of their biology and improve both diagnostics and therapeutics.

Data are presented within the following three strands:

Phage-based diagnostics was developed and validated for detecting *Borrelia* infection in patient blood samples. The performance of the LD assay was examined against 222 patient samples and showed an overall sensitivity of >90% and a specificity of 100%. This phage method has the potential of identifying active *Borrelia* infection and distinguishing between LD and RF. A national tick collecting network has been established throughout the UK, from which a screening strategy has been developed for detecting lytic *Borrelia* phages. In addition, novel phage/phage-like particles were observed from Mitomycin C-treated *Borrelia* cultures. Lytic phage hunt and temperate phage purification are ongoing. Bioinformatic and manual annotation have identified a pair of *Borrelia* phage-encoded holins (enzymes that rupture bacterial cytoplasmic membranes) and endolysins (enzymes that break down bacterial cell walls). Both have been manufactured in a yeast protein expression system and are currently under investigation of their *in vitro* 'anti-*Borrelia*' properties.

P56 Comparison of Borrelia antibody levels in serum, cerebrospinal and synovial fluid samples using various diagnostic kits and methods with different antigenic composition

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The laboratory diagnostics of Lyme disease is based on detection of specific anti-borrelia antibodies in the IgG and IgM antibody classes. To increase sensitivity of the tests, the spectrum of antigens used, mostly recombinant, is continuously extended. Several diagnostic methods (recombinant EIA, three BLOT-LINE tests of various provenance, and Microblot-Array tests with a wide range of specific antigens originating from main *Borrelia* strains) were used for testing of clinically well-defined sample sera (n=142), paired CSF samples + sera (n=104) and synovial fluid samples (n=80). The samples used were diagnosed with Lyme disease in various stages.

The primary aims were to monitor the reactivity of individual antigens (also in regards to their spectra) in relation to the type of the disease and type of the tested material, and to compare the sensitivity and specificity of the individual methods.

Extension of the antigenic spectra has proven to be a useful tool especially for the IgM class, where diagnostics is mostly based only on presence of the OspC antigen. Thus, presence of other markers may significantly contribute to positivity of the tested sample.

The obtained results have proven significant difference between the reactivity of VlsE and OspC antigens in the sera and CSF samples within the patients with neuroborreliosis, especially in the IgM class. The reactivity of the antigens from sera and synovial fluid samples was practically identical.

For verification of the specificity, a control panel was used. It consisted of samples from blood donors and patients with diagnosed tick-borne disease (n=100). In the Microblot-Array test (with 19 *Borrelia* antigens), specificities of 98% in IgG and 95.8% in IgM were achieved.

In conclusion, it was found out that extension of the antigenic spectra may lead to improved accuracy of the Lyme borreliosis diagnostics without a negative influence on the test specificity

P57 Diagnosis of Borreliosis (Lyme Disease and Relapsing Fever) in US and Europe by immune- blots

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Borreliosis is caused by two groups of Borrelia, B. burgdorferi group and the Relapsing Fever Borrelia group. Until recently it was believed that B. burgdorferi group is the only group that causes Lyme-like symptoms. However we now know that Relapsing Fever Borrelia too causes Lyme-like symptoms. Symptoms caused by both these Borrelia groups are often confused with MS, Chronic fatigue, osteo-arthritis, and ALS.Therefore it is necessary to perform appropriate diagnostic tests to differentiate Borreliosis from other diseases.Thus we have developed highly sensitive and specific immunoblots to detect and differentiate the two groups of Borrelia. The B. burgdorferi immunoblots detect antibodies to major species of B. burgdorferi sensu lato and the Relapsing Fever Borrelia immunoblots detect antibodies to Relapsing Fever Borrelia group. Based on our studies in US the specificity of the immunoblots is greater 92% for IgM and 100% for IgG. Using these immunobots we have demonstrated that both B. burgdorferi and Relapsing Fever Borrelia are present in US, Europe and Australia. The European study was performed on 310 serum samples, 149 from forest workers; 121 from patients; 10 from Lyme positive patients; and 30 from healthy controls, living in endemic and non-endemic regions for Borreliosis in Ukraine.Based on this study, 62.4% of the forest workers and 32.2% of patients had antibodies to Borrelia. In the forest workers group, 14.1% had antibodies to both, Lyme and Relapsing Fever Borrelia; 36.9% had antibodies to B. burgdorferi only and 11.4% had antibodies to Relapsing Fever Borrelia only. In the patient group, 7.4% had antibodies to both, Lyme and Relapsing Fever Borrelia; 19% had antibodies to B. burgdorferi only and 5.8% had antibodies to Relapsing Fever Borrelia only.

P58 Comparison of relative sensitivities of two commercially available tests for detection of Borrelia burgdorferi-specific antibodies

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This study compared relative sensitives of 2 commercially available tests for detection of antibodies to *Borrelia burgdorferi* (Lyme). The relative sensitivities were compared for over 40 positive patient samples at relevant end point sera dilutions. Tests under investigation included (i) the antibody capture enzyme immunoassay (EIA) test performed by the Imugen service laboratory, a division of Oxford Immunotec Inc, Norwood, MA and (ii) the Captia™ *B. burgdorferi* IgG/IgM Enzyme-Linked Immunosorbent Assay (ELISA) from Trinity Biotech.

Both tests use antigens obtained from purified *Borrelia burgdorferi* strains for the detection of *Borrelia*-specific antibodies. The EIA test uses the *B. burgdorferi* 49736 strain (a tick isolate from the upper Midwest which lacks the gene for OspA) to assess IgM, IgA and IgG antibody classes in separate assays, whereas the Captia test employs a *B. burgdorferi strain B-31* and detects IgG and IgM antibodies simultaneously, without differentiation.

Excess sera positive for the presence of *Borrelia*-specific antibodies by EIA were used. Specimen were analysed at their original dilution as recommended by the manufacturer and further diluted up to 1:500,000 to generate samples containing low levels of *Borrelia*-specific antibodies.

The EIA test showed on average a 13 fold higher relative sensitivity in detecting *Borrelia*-specific antibodies compared to the Captia test. A small subset of samples with early *Borrelia* infection were selected and assessed. Early infection was defined as IgM response only, in absence of IgG and IgA and, in some cases, also positive for Borrelia DNA by PCR *B. burgdorferi*. The EIA test had higher relative sensitivity than the Captia test in this subset of samples.

In this study, the antibody capture EIA test by Imugen detected *Borrelia*-specific antibodies at a higher dilution than the Capita test. This higher relative sensitivity suggests that the EIA test may detect antibodies that would have otherwise been missed if tested by other ELISAs.

P59 Multiplex Lyme IgG and IgM assays for improved detection of early Lyme Disease in a European endemic population

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The increased incidence of Lyme borreliosis over the last decade in conjunction with variations in clinical manifestations of disease has highlighted the need for improved diagnostics. Lyme Borreliosis is diagnosed by a combination of signs and symptoms supplemented with serological results. The efficacy of serology is dependent on multiple factors including target antigens and methodology. The objective of this study was to identify biomarkers that improve sensitivity in early Lyme disease while maintaining high specificity. A total of 138 peptides, recombinant full-length proteins, and whole cell lysates were screened, using Luminex technology, in a cohort of endemic European samples. The biomarker panel consisting of sequences derived from the major Borrelia species present in Europe, including B. afzelii, B. garinii, B. spielmanii, and B. burgdorferi sensu stricto, was screened for Lyme IgG and Lyme IgM assays.

A single dipeptide consisting of sequences from Borrelia Flagellin B and VlsE proteins yields a positive agreement of 99.0% against predicate tested samples with a specificity of 96.6% when monitoring for an IgG response. In early Lyme samples with confirmed erythema migrans rash, assay sensitivity was 88.2% using this single marker. The addition of two more markers to the IgG assay, recombinant B. garinii p58 and B. afzeliiDbpA, resulted in a positive agreement of 100% with a specificity of 94.0%. Addition of the extra markers showed no improvement on assay sensitivity for early Lyme samples.

For IgM detection, combining B. burgdorferi OspC and B. garinii VlsE resulted in a positive agreement of 64.2% in predicate tested samples with a specificity of 96.2%. The addition of B. garinii as a third marker increased the positive agreement to 70.0% with a specificity of 94.2%. Sensitivity in early Lyme samples using a 2 or 3-marker IgM panel was 33.3% and 73.3% respectively.

P60 Evaluation of a new microarray for detection of Borrelia burgdorferi specific antibodies in human serum samples

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Background Laboratory diagnosis of Lyme borreliosis is mainly based on detection of specific antibodies in serum or liquor. A two-step algorithm comprising a sensitive screening and a subsequent confirmatory test (e.g. Immunoblot using native and/or recombinant antigens) is recommended.

Material/methods 140 serum samples from clinically defined patients:Erythema migrans (EM, n= 66), neuroborreliosis (NB, n=35), Acrodermatitis chronica atrophicans (ACA, n=19) and Lyme arthritis (AT, n= 20).
Specificity controls included sera from: healthy blood donors (n=97), pregnant women (n=50), rheumatoid factor (RF) positives (n=9) and TPPA positives (n=11).
A new microarray (*SeraSpot*® Anti-Borrelia IgG/IgM, Seramun Diagnostica GmbH, Heidesee, GERMANY) was evaluated with respect to sensitivity and specificity of IgG and /or IgM antibody detection. The test uses recombinant antigens (VlsE, p39, p58, p100, OspC, DbpA) spotted into 96-well microtitration plates. Image analysis and result interpretation are performed by a reader and corresponding software.

Results Compared to clinical diagnosis the sensitivity (positive results only) of *SeraSpot*® Anti-Borrelia was determined 42.4% (IgG) and 66.7% (IgM) for the EM group, 91.4% (IgG) and 60.0% (IgM) for the neuroborreliosis group, 100% (IgG) and 31.6% (IgM) for the ACA group and 100% (IgG) and 35% (IgM) for the AT group. Summarizing IgG and IgM detection the sensitivity was 87.9% (EM), 97.1% (NB), 100% (ACA) and 100% (AT).
The specificity in the control groups was calculated 94.8% (IgG) and 95.9% (IgM) for healthy blood donors, 90.9% and 81.8% resp. for the *T. pallidum* positive samples, 100% and 100% resp. for the RF positive samples and 100% and 86.0% resp.for the samples from pregnant women.

Conclusions The new microarray *SeraSpot*® Anti-Borrelia IgG/IgM is a sensitive and specific method suitable for the serodiagnosis of Lyme borreliosis. The excellent performance characteristics combined with standardized result interpretation might be beneficial in comparison to commonly used methods.

P61 Comparison of LIAISON and Luminex tests for IgM and IgG detection in patients with Lyme borreliosis

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Background Detection of specific antibodies is the most frequently used microbiological method for confirming Lyme borreliosis (LB). The aimof the study was to compare two serological tests for IgM and IgG detection in blood of patients with LB, and to determine the presence of antibodies to particular borrelial antigens.

Material and methods 28 serum specimens (20 obtained from patients with early LB, 8 from control group) were tested with two tests: LIAISON (utilizing antigens OspC and VlsE for detection of IgM antibodies, and VlsE for IgG antibodies) and LUMINEX (utilizing antigens p100, VlsE, p58, p39, OspA, OspC and p18 for detection of IgM and IgG antibodies).

Results Identical results of both tests for IgM and IgG (either positive, borderline or negative) were obtained in 22/28 (78.6%) samples, respectively. LIAISON was slightly more sensitive for IgM and IgG but less specific for IgM. Completely divergent results were established for three IgM and one IgG sample (two IgM and IgG sample were positive with LIAISON but negative with Luminex, and one IgM vice versa). All 9 Luminex IgM positive samples reacted with three OspC antigens; IgM to VlsE was detected in two samples only (positive and borderline, respectively). All 13 Luminex IgG positive and borderline samples reacted with VlsE and p58 antigen, 8 (61.5%) with p18 *B. afzelii*, 6 (46.1%) with p100, while the proportion of antibodies to other antigens was even lower.

Discussion and conclusions The present study indicates that VlsE antigen is suitable for detection of specific borrelial IgG antibodies, and OspC antigen for detection of specific IgM antibodies. Although Luminex contains additional antigens, this supplement does not influence the sensitivity of antibody detection. LIAISON test is performed nearly completely automatically while Luminex test requires a lot of manual work.

P62 Improving diagnosis of Borrelia infection through identification of Borrelia-specific T cells

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Diagnosis of Lyme disease patients is challenging due to lack of predictive biomarkers. We are investigating the diagnostic potential of measuring Borrelia-specific T cells in blood.

A biobank of 300+ blood samples from borrelia-infected patients and healthy control subjects was established by collecting blood from subjects in three endemic areas in Åland, Norway and Poland and in Denmark over a period of 3 years. From each sample serum and PBMC's were isolated and stored. All samples were HLA-typed and serum tested for Borrelia serology. Patients with Erythema migrans, Neuroborreliosis and Lyme arthritis were included. The diagnosis was based on evaluation of clinical and laboratory criteria according to Stanek et al.: European Lyme borreliosis case definitions and Mygland Å et al.:EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis.

MHC Dextramers displaying Borrelia peptides were used to directly measure Borrelia-specific T cells in blood using flow cytometry. Peptides able to bind HLA-A*0201 were predicted from 40 different Borrelia-specific antigens using NetMHC 4.3. Based on these predictions a library of 253 Borrelia-specific Dextramers were generated and screened in pools of up to 12 Dextramers on a set of HLA-matched PBMC samples from the biobank. Borrelia-specific T-cell responses undetectable in healthy control samples were measurable in seropositive samples from patients with EM or neuroborreliosis diagnosis.

The 40 most promising Dextramers were screened individually to verify their identification of Borrelia-specific T cells in patient samples. Each Dextramer were analyzed on samples from 2-4 patients with late state active disease and on 3+ healthy control samples. 12 of the Dextramers identified Borrelia-specific T cells in 25-100% of the patient samples they were tested on and did not measure such T-cell responses in healthy control samples.

P63 The cost of Lyme borreliosis

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Background Lyme borreliosis (LB) is the most frequently reported tick-borne infection in Europe and North America. The aim of this study was to estimate the cost-of-illness of LB in the Netherlands.

Methods We used available incidence estimates from 2010 for tick bite consultations and three symptomatic LB outcomes: erythema migrans (EM), disseminated LB and Lyme-related persisting symptoms. The cost was estimated using these incidences and the average cost per patient as derived from a patient questionnaire. We estimated the cost from a societal perspective, including healthcare cost, patient cost and production loss, using the friction cost method and a 4% annual discount rate.

Results Tick bites and LB in 2010 led to a societal cost of €19.3 million (95% CI: 15.6-23.4; 16.6 million population) for the Netherlands. Healthcare cost and production loss each constituted 48% of the total cost (€9.3 million/year and €9.2 million/year), and patient cost 4% (€0.8 million/year). Of the total cost, 37% was related to disseminated LB, followed by 27% for persisting symptoms, 22% for tick bites, and 14% for EM. Per outcome, for an individual case the mean cost of disseminated LB and Lyme-related persisting symptoms was both around €5,700; for EM and GP consultations for tick bites this was €122 and €53. As an alternative to the friction cost method, the human capital method resulted in a total cost of €23.5 million/year.

Conclusion To our knowledge, this is the first cost-of-illness estimate for LB that includes all possible health outcomes, including tick bites and Lyme-related persisting symptoms. LB leads to a substantial societal cost. This calls for additional preventive measures, of which the cost-effectiveness can be evaluated using the results of this study.

P64 Lyme borreliosis as “the overlap syndrome”

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Aim: the presentation of Lyme borreliosis (LB) cases, as “the overlap syndrome" in patients with other long lasting pathological conditions.

Cases reports: 1/ In a 69-year-old man with multiple sclerosis *(SM)*, with the right lower extremity (r.l.e.) palsy, two years before the admission to our department progression of the palsy and paresis of the right upper extremity (r.u.e.) was noted. In subsequent months arthritis was also observed. Despite the anamnesis of a tick bite and *erythema migrans (EM)* preceding the above symptoms, the two-tiered laboratory serum testing algorithm (ELISA and Western blot) was performed only after many months and confirmed *Borrelia garinii (B.g.)* infection. Cerebrospinal fluid (CSF) examinations revealed lymphocytic pleocytosis, the increased protein level and the positive *B.g.* CSF/serum antibodies index, with the negative result of CSF *Borrelia burgdorferi* s.l. PCR test. Ceftriaxone i.v. treatment resulted in the complete withdrawal of the r.u.e. paresis, the partial withdrawal ofthe r.l.e. paresis and the withdrawal of arthritis.

2/ In a 55-year-old man with rheumatoid arthritis, the increase of arthritis activity with involvement of lower extremities large joints had been observed for three years before the admission to our department. Simultaneously, symptoms of bilateral brachial plexus palsy, transforming into paresis of the shoulder girdle muscles, suggesting Parsonage-Turner syndrome, had started. Similarly like in the previous case, borrelial infection was not considered for months. Performed ELISA and Western blot serum tests as well as CSF assays revealed results similar to findings obtained in the patient with *SM*. Ceftriaxone i.v. therapy resulted in the complete withdrawal of the knees and ankles arthritis and significant recovery of bilateral brachial plexopathy.

Conclusion: The correct diagnosis and treatment of LB “overlapping" on neurological and musculoskeletal system diseases may diminish disability and exert essential influence on the patients’ quality of life.

P65 Dissecting the human immune response toward Borrelia

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Borrelia is one of four genera within the phylum of Spirochaetes and holds the agents for both relapsing fever and Lyme Borreliosis (LB). In Europe, the genospecies *B. afzelii*, *B. bavariensis, B. burgdorferi* sensu stricto (s.s.), *B. garinii* and *B. spielmanii* are known to cause LB. In the United States *B. burgdorferi* s.s. is - to the largest part - the sole agent of LB. Humans can act as accidental hosts when spirochetes are transmitted during the bite of an infected tick. *Borrelia* possess various mechanisms to evade the human immune system. This project aims to shed light on the complex and largely unknown interaction of *Borrelia* with the human immune system.

Hence, various *Borrelia* strains were grown to logarithmic growth phase. Subsequently, we co-cultivated them with human umbilical vein endothelial cells. In order to observe the interactions by fluorescence microscopy we stained *Borrelia* with carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) and counterstained human cells with 4',6-diamidino-2-phenylindole (DAPI) and Alexa 568 Phalloidin. Major differences could be detected between the strains as some were rapidly internalized while others additionally showed massive adhesion to cell surface. Therefore, the plasmid content was analyzed by multiplex PCR as differences detected in genomic structure allows to pinpoint variances during co-cultivation. Furthermore, this method will provide an important tool studying the plasmid content regularly.

The next steps will be to co-cultivate various *Borrelia* strains with M1/M2 macrophages. Subsequently transcriptome profiling is planned to gain comprehensive insight. Moreover, we plan analysing signal translation from innate immune cells toward an adaptive immunity response: T cell proliferation and differentiation will be in the focus. These studies should provide insight why *Borrelia* are able to frequently escape the immune system. Moreover, we hope to identify novel biomarkers to differentiate between acute and chronic infections.

P66 Attachment of Borrelia burgdorferi to Vero cells

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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 adhereto human and murine fibroblasts, endothelial cells, epithelial cells, macrophages, neuronal and glial cells, fibrocytes, and lymphocytes; also tick cells, and Vero cells.

At present there is a gap in the understanding of infection dynamic proceses such as intravascular transport of the borrelia, mechanisms of borrelia escape from the vascular network and possible role of cell components involved in organotropism. Presence of the variety of the substances on the pathogen cell surface substantiates chemical and physical properties of the cell such as surface charge, hydrophobicity/hydrophilicity, antigenicity/immunogenicity.These properties have a profound impact on the activity of bacterial cells to adhere to host cell receptors, to interact with host cells and to colonise the different loci in the host.

We investigated by electronmicroscopy the adherence of the long-term in vitro passaged motile *Borrelia burgdorferi* to primate kidney epithelial Vero cells. These cells have been shown as an interesting model for study of the toxic potential of many bacterial pathogens. We have focused to the intitial phase of the interaction between *Borrelia burgdorferi* and Vero cells. A vertical contact between borreliae and Vero cells was confirmed already after one hour of incubation at 37 0C, 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 in vitro 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.

P67 Design of a prospective study on Lyme borreliosis and other tick-borne (co-)infections in patients with an erythema migrans or fever after a recent tick-bite in Belgium

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Besides *Borrelia Burgdorferi* s.l., several other tick-borne pathogens have been found to circulate in Belgian ticks. However, little is known about the diseases they might cause in humans and their role as a co-infection in patients with Lyme borreliosis. As part of a larger project (Humtick) a study is set up to evaluate the occurrence and clinical presentation of the following pathogens in the Belgian human population: *Anaplasma phagocytophilum*, *Borrelia miyamotoi*, *Candidatus* Neoehrlichia mikurensis, *Babesia* spp. and *Rickettsia* spp. . In addition, the study will analyze the role of co-infections as a possible risk factor for the development of Post Treatment Lyme Disease Syndrome (PTLDS) in patients with an erythema migrans (EM). Since June 2016 and up to August 2018, about 600 patients with an erythema migrans and 200 patients with fever after a recent tick-bite (< 1 month) are included in the study, through a network of 200 general practitioners. At inclusion, data on health status, diagnosis and treatment are collected through a questionnaire filled in by the GP and the patient, and a blood sample is collected. These samples will be tested for the presence of tick-borne pathogens using multiplex polymerase chain reaction (PCR) techniques. The incidence of PTLDS in EM patients will be estimated using standardized questionnaires on health status and impact on quality of life, repeated during a follow-up period of 6 to 24 months. Results will be compared with self-reported pre-Lyme health status, a control group and existing Belgian population norms. At this stage, the methodology of the study will be presented. Results of the study are expected by the end of 2018.

P68 Human infection with Rickettsia raoultii in Slovakia

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Rickettsia raoultii, *Rickettsia* sp. genotypes DnS14, DnS28 and RpA4, was first identified as new rickettsiae of the *Rickettsia massiliae* genogroup, by 16S rDNA, gltA and ompA sequencing from *Dermacentor nutallii* ticks collected in Siberia and *Rhipicephalus pumilio* ticks collected in Astrakhan in 1999. *R. raoultii* strains were isolated from PCR-positive *Dermacentor* ticks, *Dermacentor silvarum*, *D. nuttalli*, *Dermacentor reticulatus* and *Dermacentor marginatus*, collected in Russia, Kazakhstan, France and Slovakia using cell cultures. In 2008, it was identified as a novel **Rickettsia** species on the basis of its genetic and serologic characteristics. Since 1999, *R. raoultii* has been detected in *Dermacentor* ticks throughout Europe, in the European part of Russia, Germany, Spain, Portugal, Netherlands, Slovakia, France, Croatia, Poland, UK and in some parts of Asia, and in *Haemaphysalis punctata* collected in Spain. Infection rates in Europe range from 2% to 80%. In 2002, *R. raoultii* DNA was detected in *D. marginatus* tick removed from the scalp of a patient in whom TIBOLA (tick-borne lymphadenopathy)/DEBONEL (Dermacentor-borne necrosis erythema and lymphadenopathy) developed in France. Human infection with *R. raoultii* have also been reported in Hungary, Italy, Spain, China and now in Slovakia. Seven year old girl had been bitten by a tick on her head. Rash, lymphadenopathy and fever were developed. The presence of *R. raoultii* was confirmed in *D. reticulatus* female taken from the scalp of this patient and *Rickettsia sp.* in serum specimen using molecular methods. Serum specimens were tested by ELISA assay and the presence of IgM antibodies against spotted fever group rickettsiae was confirmed. Oral clarithromycin was prescribed.

Slovakia is a country where vector of *R. raoultii* - *D. reticulatus* and *D. marginatus* ticks commonly occur on the vegetation, thus represent risk for human infection.

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P69 Diversity of rickettsiae and rickettsial endosymbionts in ectoparasites from small mammals

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Rodents are important hosts of ectoparasites such as ticks, fleas and mites and important reservoirs of zoonotic pathogens such as bartonellae and borreliae, but their reservoir potential for rickettsiae is still discussed. In this study, the aim was to analyze the diversity of rickettsiae and rickettsial endosymbionts present in ectoparasites feeding on small mammals trapped in sylvatic and urban habitats of Eastern Slovakia between 2008 and 2013. For surveillance of rickettsial diversity methods based on PCR amplification of *gltA*, *ompB* and 23S rRNA genes and sequencing were employed. Dominant rodent species were *Apodemus agrarius*, *Apodemus flavicollis* and *Clethrionomys glareolus*. *Ixodes ricinus* and *Ixodes trianguliceps* were tick species removed from hosts. The most prevalent flea species were *Ctenophthalmus agyrtes*, *Ctenophthalmus solutus* and *Megabothris turbidus*. *Laelaps agilis, Haemogamasus nidi, Myonyssus gigas* and *Eulaelaps stabularis* were common mite species occurring on small mammals. Rickettsiae found in ectoparasites and ear biopsies of rodents belong to *R. helvetica, R. raoultii* and *R. slovaca* genotypes. Ticks are known vectors of these rickettsial species. Based on *gltA* genes some sequences from mites and biopsies of rodents belong to the clade of *Rickettsia* sp. MG116-4 previously identified in mite from Korea, and *Candidatus* R. senegalensis identified in *C. felis* from Senegal and India. The exact role of mites and fleas in the transmission of these species of rickettsiae is unknown. Furthermore, in rodent biopsies rickettsiae similar to endosymbionts of *Dolichopus nubilus* and *Chrysotus neglectus* were found.

The results of the study suggest circulation of rickettsiae and rickettsial endosymbionts between small mammals, fleas, mites and ticks in studied localities.

The study was supported by projects VEGA 2/0068/17 and VEGA 2/0059/15.

P70 The inhibitory effect of mint essential oils on growth of *Rickettsia slovaca*

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The aim of the present study was to estimate the inhibitory effect of two mint essential oils, peppermint (*Mentha* x *piperita*) and spearmint (*M. spicata*) on the growth of *R. slovaca*. Monolayers were infected by 10^6 or 10^8 rickettsiae and cultivated with 0.01 % mint oils (dose non-cytotoxic for Vero cells) since the first day post infection (p.i.). Intracellular growth of *R. slovaca* was evaluated by quantitative PCR. Compared to controls slighter and no discernable cytopathic effect was observed in cells treated with spearmint and peppermint oil on the fourth day p.i., respectively. Spearmint oil decreased the growth of *R. slovaca* in cells infected by 10^6 rickettsiae to 61.57, 16.06, and 6.71 % of mean number assessed in controls; and in cells infected by 10^8 rickettsiae to 16.73, 20.30 and 24.55 % on days 2, 3 and 4 p.i., respectively. Average number of *R. slovaca* particles in peppermint oil treated cells infected by 10^6 rickettsiae equaled to 1.88, 0.09, and 0.05 % of controls. Treatment with peppermint oil decreased the rickettsial load in cells infected by 10^8 rickettsiae to 1.14, 0.17 and 0.18 % of controls on days 2, 3 and 4 p.i., respectively. Linear models on log transformed data from days 2-4 p.i. confirmed that 0.01 % peppermint oil significantly interfers with the qrowth of *R. slovaca* ($p < 0.001$). Reduced number of *R. slovaca* particles cultivated with this dose of spearmint oil was significant only on day 4 p.i. in cells initially infected by 10^6 rickettsiae ($p=0.031$). Our results suggest that essential oils of some culinary plants known for their antimicrobial properties may also inhibit intracellular tick-borne bacteriae such as *R. slovaca*.

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P71 First cases of molecular detection of Rickettsia raoultii and Rickettsia aeschlimannii in patients in Russia

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Tick-borne rickettsioses are infectious diseases transmitted to humans by ixodid ticks. Until recently, Siberian tick typhus (STT) caused by *Rickettsia sibirica* was considered the onlytick-borne rickettsiosis in the Asian part of Russia; however, only a few cases of rickettsioses have been genetically confirmed. To establish etiologic agents of tick-borne rickettsioses, blood and cerebrospinal fluid samples from 348 patients hospitalized in the Novosibirsk Municipal Clinical Hospital of Infectious Diseases No. 1 in 2016-2017 were examined for the presenceof *Rickettsia* spp. DNA with subsequent sequencing of revealed DNA-fragments. DNA of three rickettsial agents was identified in examined samples. *R. sibirica* subsp. *sibirica* DNA was detected in 7 (2.0%) patients, *Rickettsia raoultii* DNA was found in 4 (1.1%) patients, and *Rickettsia aeschlimannii*, in one patient. *R. raoultii* isolates belonged to three different genetic variants: DnS14, RpA4 and a new one. Most patients with *R. sibirica* infection and patient with *R. aeschlimannii*infection had typical symptoms of STT. However, one patient with *R. sibirica* infection had no rash or eschar, which led toamisdiagnosis. Clinical manifestations of rickettsiosis caused by *R. raoultii* varied and significantly differed from that of STT. None patients with *R. raoultii* infection presented with maculopapular rashes, eschars, arthralgia, or myalgia. Most patients with *R. raoultii* infection had low-grade fever; among them, one patient had erythema and one demonstrated a short episode of meningeal syndrome. One patient had high-grade prolonged fever, regional lymphadenopathy and single elements of the rash around the site of the tick bite, but he additionally had AIDS. Notably, *Rickettsia* spp. DNA was found in several patients without typical STT signs; this allows us to state that patients with atypical symptoms should be properly investigated on the presence of *Rickettsia* infection. This study was supported by the Russian Scientific Foundation, research project no. 15-14-20020.

P72 Detection of CRISPR / Cas structures in the genome of the Rickettsia bellii strain by bioinformatics methods

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Rickettsia are intracellular bacterial parasites causing rickettsiosis. Until now, there is no data on the detection of CRISPR / Cas-systems in the rickettsia genomes. In this work, through the software methods of bioinformatics, it was possible to identify such structures in the genome of the strain *Rickettsia bellii* OSU 85-389 (GenBank No. NC_009883). To search for CRISPR / Cas- systems, the methods from the package MacSyFinderver were used. 1.0.2. The search for genes of cas-proteins was carried out by methods from the package program makeblastdb ver.2.2.28 and HMMER ver.3.0. To search for CRISPR cassettes in the genome was used an algorithm which included 5 programs: 1) PILER-CR; 2) CRISPI; 3) CRISPRFinder, 4) CRT; 5) CRISPRDetect. Screening of phages through the spacers of CRISPR cassettes was carried out using the BLASTn search algorithm for the GenBank-Phage database, using the programs: CRISPRTarget, Mycobacteriophage Database, Phages database. Only two cas-genes were identified - csf4_TypeU. In the genome, two CRISPR cassettes were also found. In the first CRISPR cassette, four spacer sites were identified, separated by repeats of 35 nb. The second CRISPR cassette had 8 spacers separated by repeats of 23 nb. Through the spacers of the first CRISPR cassette, complementary areas (protospacers) of phages of bacterial genera *Gordonia, Arthrobacter, Rhodococcus* measuring 14-15 nb were identified. Through the spacers of the second CRISPR cassette, protospacers of phages, belonging to the bacteria of the genera *Streptomyces, Rhodococcus Arthrobacter, Gordonia, Mycobacterium* 14 to 22 nb long were identified. Thus, the bioinformation programs used have shown the possibility of searching for CRISPR / Cas structures in the bacterial genomes, and also through the spacers of their CRISPR cassette to identify the phagotypes with which the given bacterium have contacted in its evolutionary history.

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P73 Prevalence of infection with Rickettsia helvetica in Ixodes ricinus ticks feeding on non-rickettsiemic hedgehogs (Erinaceus europaeus) in sylvatic habitats of west-central Poland

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The European hedgehog, *Erinaceus europaeus*, is frequently parasitized by *Ixodes ricinus* being main vector of TBE virus, *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, *Rickettsia* spp. and others. To date, however, only limited information has been available on the role of the hedgehog as a host or reservoir for these pathogens in Europe.

We investigated blood samples and ixodid ticks from European hedgehogs for the presence of *Rickettsia* spp. A total of 70 blood samples, 716 *Ixodes ricinus* and 5 I. hexagonus were collected from 70 hedgehogs life-trapped in sylvatic environment in western Poland in 2012-2014. Samples were tested by a conventional PCR assay with primers targeting gltA gene of 165 bp. Female ticks were tested individually, larvae and nymphs in pools (2-5 ticks). None of blood samples and *I. hexagonus* was PCR positive for Rickettsia spp.while rickettsial DNA was found in at least 9.3 *I. ricinus*, with an increase detected with each tick stage (7% - larvae, 8.9% - nymphs and 19.8% - females). Temporal and seasonal differences in Rickettsia prevalence were observed, being the highest in 2012 (11.5%) and in the autumn (28.8%), respectively.

Positive samples were subjected to nested and semi-nested PCRs targeting the partial *ompA* and 16s rRNA genes, respectively. All samples examined by the nested PCR were negative, what suggested that ticks were infected with *Rickettsia helvetica,* asthe *ompA*-gene seems to be not amplified in this species. Using sequencing of partial 16S rRNA, R. helvetica was the only species identified.

These results indicate that European hedgehogs are exposed to R. helvetica via infected ticks and might be involved in its natural transmission cycle also as a potential vertebrate reservoir for thisspecies.

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P74 Transmission of Rickettsia raoultii and Rickettsia massiliae by in vitro feeding Dermacentor reticulatus and Rhipicephalus sanguineus ticks

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Dermacentor reticulatus is considered to be one of the main vector ticks for *Rickettsia raoultii,* whereas *Rhipicephalus sanguineus* is recognized as a potential vector of *Rickettsia massiliae*. The aim of this study was to investigate the transmission of Spotted Fever Group Rickettsiae (SFGR) by *in vitro f*eeding *D. reticulatus* and *R. sanguineus* ticks and determine whether SFGR are maintained through subsequent generations of ticks.

D. reticulatus and *R. sanguineus* ticks were allowed to feed through silicone membranes *in vitro.* During tick feeding, the blood meal was examined by PCR/Reverse line blot hybridization (RLB) at regular intervals for evidence of SFGR transmission. After feeding, all ticks were also tested by PCR/RLB.

Moreover, hemolymph samples and/or ticks from a laboratory colony of *D. reticulatus* ticks originating from the 1st and the 5th generation were tested for the presence of SFGR.

D. reticulatus ticks used in the *in vitro* feeding experiments were infected with *R. raoultii* (71.1%) and were able to transmit *R. raoultii in vitro*. *R. massiliae* was detected in *R.sanguineus* ticks which transmitted *R. massiliae in vitro* as early as 8h post application. Moreover, nearly 50% of *D. reticulatus* ticks randomly selected from the colonies were positive for *R. raoultii*, whereas 80% of hemolymph samples were positive for *R. raoultii.*

This study presents for the first time the ability of *D. reticulatus* and *R. sanguineus* ticks to transmit *in vitro R. raoultii* and *R. massiliae*, respectively. The high infection rate of *R. raoultii* in the hemolymph suggests that the infection is systemic and maintained in at least five laboratory generations of *D. reticulatus* ticks.

P75 Molecular detection of tick-borne microbes within ticks collected from hosts in Zimbabwe

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On a global scale, ticks and tick-borne pathogens pose large health risks for humans and animals and cause significant economic losses especially in developing countries.

To assess the current presence of tick-borne microorganisms within Zimbabwe, a pilot study was carried out in which 254 ticks were collected from different hosts (i.e. cattle, dogs, horse, goat and a lion) in different geographical locations. These ticks consisted of several species namely: *Amblyomma hebraeum*, *A. variegatum*, *Haemaphysalis leachi*, *Hyalomma marginatum rufipes*, *Hy. truncatum*, *Rhipicephalus appendiculatus*, *R. decoloratus*, *R. evertsi*, *R. microplus*, *R. sanguineus* and *R. simus*.

Up to five ticks per tick species per host were selected resulting in a total number of 145 ticks which were used for molecular analysis using the reverse line blot. DNA was extracted from these ticks and PCRs were carried out using primers targeting the following genera: *Anaplasma*, *Babesia*, *Borrelia* sensu lato, *Ehrlichia*, *Rickettsia* and *Theileria* using a biotinylated reverse primer. Obtained PCR fragments were hybridized, using a miniblotter, onto a Biodyne® C membrane on which genus and species-specific probes were covalently linked. Hybridized products were visualized by chemiluminescence using a streptavidin-horse radish peroxidase conjugate and ECL substrate.

Within the analysed ticks the DNA was detected of the following microorganisms: *Anaplasma marginale*, *Babesia caballi*, *Ehrlichia canis*, *Ehrlichia ruminantium*, *Rickettsia aeschlimannii*, *Rickettsia conorii*, *Theileria mutans*, *Theileria taurotragi* and *Theileria velifera*. Genus specific catch-all signals, without a species-specific signal, were subsequently sequenced using non-biotinylated RLB-PCR primers to identify the respective microorganism.

The results obtained during this pilot study reveal a significant risk for humans and animals in Zimbabwe. A follow up study is suggested to accurately assess the prevalence of tick-borne microorganisms in the different provinces of Zimbabwe.

P76 Detection of Ehrlichia, Candidatus Neoehrlichia and Rickettsia spp. in Estonian ticks

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Ticks are capable of transmitting a wide range of infectious agents, that in addition to causing a severe economic loss in livestock industries worldwide, also greatly impact public health. Moreover, in the last decades, the incidence of tick-associated diseases has increased globally, making tick-borne infections an emerging threat with high morbidity and mortality.

The most common tick-borne diseases in Northern Europe, Tick-Borne Encephalitis and Lyme borreliosis, are endemic in Estonia, with on average 146 and 1384 cases reported annually, respectively. However, besides the well-known causative agents for these diseases, new potentially pathogenic species, strains or genetic variants are being detected in ticks and tick-bitten patients constantly. To assess the possible growing health risks for the general population, we analysed the presence of *Rickettsia*, *Ehrlichia* and *Candidatus* Neoehrlichia spp. in Estonian ticks.

In the current study, 1640 ticks collected from 5 geographical regions of Estonia were screened for the presence of *Rickettsia* spp., and 776 ticks from 7 counties were analysed for the presence of *Ehrlichia* and *Candidatus* Neoehrlichia species. Three potentially pathogenic *Rickettsia* spp. were found – *R. helvetica*, *R. monacensis* and *Candidatus* R. tarasevichiae, with an overall prevalence of 5,1% across the study sites. *Ehrlichia muris* was detected in three counties, with the site-specific prevalence varying from 1,2% to 25,6%. The study also revealed the presence of *Candidatus* N. mikurensis in the western regions of Estonia, with the general prevalence of 0,9%.

Altogether, five new potentially pathogenic bacteria species were identified circulating in Estonian ticks, that the physicians and the general public of Estonia should be aware of. Together with the prevalence of TBEV and BBSL in Estonian tick populations, we conclude that there is a high risk of obtaining a tick-borne infection or coinfection after a tick bite in Estonia.

P77 Molecular detection of Babesia and Theileria species in Australian ticks
withdrawn

P78 Molecular screening for Midichloria in hard and soft ticks reveals different prevalences and horizontal transmission

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The family *Midichloriaceae* is a clade within the order *Rickettsiales* (which includes the etiological agents of important diseases and symbionts of arthropods and nematodes). In ecological terms, this new family is possibly even more complex than the sister families *Rickettsiaceae* and *Anaplasmataceae* and its hosts range from protozoa to parasitic arthropods (ticks, fleas, bedbugs). *Midichloria mitochondrii*, symbiont of the sheep tick *Ixodes ricinus*, was the first described member of the family *Midichloriaceae*, with a prevalence of 100% in females and ~50% in males. Most *M. mitochondrii* bacteria are localized in the cells of the ovary, either in the cytoplasm or within intermembrane space of the mitochondria. The bacterium is vertically transmitted from the mother to the offspring; nevertheless, there are some molecular and serological evidences suggesting that transmission to the vertebrate host can occur after the tick blood meal. Recent reports are expanding the view of *Midichloriaceae*, now including numerous bacteria of biological and medical interest, indicating a widespread distribution with an increasing range of hosts, with ticks being highly represented. A molecular screening on 17 tick species was performed, detecting and quantifying bacteria of the family *Midichloriaceae* in nine of them, including the first report of a representative of this family in a soft tick (Argasidae), *Ornithodoros maritimus*. Based on sequence identity and phylogenetic analysis we propose that all these bacterial symbionts of ticks could be members of the genus *Midichloria*. The performed screening highlights different prevalenes and variable bacterial loads in different tick species including one, *Ixodes aulacodi*, where the bacterium is present in all examined individuals, like in *I. ricinus*. The obtained results prompt us to hypothesize different roles of *Midichloria* and the horizontal spread of these bacteria amongst ticks.

P79 Reverse Line Blot-based detection approaches of microbial pathogens in Ixodes ricinus ticks collected in Austria and impact of the chosen method

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Various detection methods based on molecular biology are currently available for the detection of numerous tick-borne pathogens in ticks, which are capable of causing tick-borne diseases in humans and animals.

During this study 554 *I.ricinus* ticks were screened for pathogens of the genera *Borrelia*, *Rickettsia*, *Anaplasma*, (*Neo*-)*Ehrlichia*, *Babesia* and *Coxiella* by using the reverse line blot (RLB) hybridization method. Compared to other methods the RLB is a very useful tool for performing semi-high throughput epidemiological studies for relatively low costs.

The RLB consists of two major steps: firstly, a PCR with a biotinylated primer is performed, resulting in biotinylated amplicons. Secondly, those amplicons are hybridized to a membrane containing genus and species specific oligonucleotide probes. A successful hybridization is then visualized by chemiluminescence.

Aside from screening for tick-borne pathogens, two method comparisons for the detection of *B.burgdorferi* sensu lato and *Rickettsia* spp. in ticks were performed, which were all based on the RLB method. For the detection of *Borrelia* the difference in the methods consisted only in the primer pairs used for PCR, while for *Rickettsia* spp. two completely independent RLBs based on different target genes were used.

Initial sensitivity testing with defined dilution series for the *Borrelia* and *Rickettsia* RLBs did not result in major differences. However, when applied to 'real' tick samples the results were highly interesting. The overall prevalence for *Borrelia* differed significantly (25.6% vs. 13.5%) and certain genospecies were better or worse detected by the different methods. This was also seen in the different *Rickettsia* RLBs.

These results show that similar methods, even if they have a similar sensitivity in the evaluation process, can lead to different prevalences of genera and species detected. Therefore, detection methods should always be chosen wisely and interpretation of results should be done with special consideration of the detection method used.

P80 Prevention of tick-borne diseases in northwestern Italy: diagnostic approach on ticks collected from humans

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Ticks are arthropods with medical and veterinary importance related to their capacity of transmitting pathogens to animals and humans.

The present study describes the surveillance system enforced in Piedmont region (Northwestern Italy), since 2011 on tick borne diseases, according to the One Health concept. An information network was activated between veterinary services and local human health authority and a communication campaign carried out to raise citizen awareness about problems associated with tick bites and to provide practical prevention advices. 1084 ticks collected from bitten humans were submitted to the laboratories of IZSPLVA, identified to species level and tested for the detection of pathogens [*Borrelia burgdorferi* sl; *Rickettsia* spp. and *Anaplasma* spp]. Most tick bites (74%) were reported between May and July, with a peak level (30%) in June. The life stages most frequently collected were nymphs (56.1%) and adult females (40.2%), followed by larvae (2.5%) and adult males (1.1%).

The tick species most commonly retrieved belonged to the *Ixodes* genus (95%), mainly identified as *Ixodes ricinus* (83%).

Moreover 20 ticks belonged to the *Riphicephalus* genus (including 5 *Rh. sanguineus*), 2 individuals to the *Haemaphysalis* genus (1 *H. concinna*; 1 *H. punctata*) and 7 *Dermacentor marginatus* were identified.

In some cases, ticks were identified only at the genus level (16,3%) or the morphological identification was not possible (2%) since the removal not properly performed. Preliminary biomolecular tests on 577 ticks showed an infection prevalence of 8.7% for *Borrelia burgdorferi* sl, 17.8% for *Rickettsia* spp. and 2.1% for *Anaplasma* spp. Fourteen ticks were co-infected by more than one pathogen.

This study indicates that tick bites are frequently reported by humans and thanks to the information campaign, preventive measures were rightly applied by citizens. This diagnostic approach could provide useful information to physicians addressing rapid diagnoses and treatment decisions.

P81 Tick-borne diseases in Russia

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The territory of Russia is a big area of natural foci diseases, including tick-borne diseases, which is 50% for all the amount of natural foci diseases. The most considerable diseases among them are Ixodes tick-borne borellioses (ITBB), tick-borne encephalitis (TBE), Crimean-Congo hemorrhagic fever (CCHF). Furthermore, in the territory of the country there are natural foci of rickettsioses, human monocytic ehrlichiosis (HME), human granulocytic anaplasmosis (HGA), Astrakhan spotted fever (ASF), which results in these tick-borne diseases amongst local population.

In order to evaluate the grade of contacts between ticks and humans, the registration of tick bite's number was introduced. The average number of tick bites was 400000-550000 cases annually, with 23-25% affecting kids.

ITBB is the most widespread from all the tick-borne infections group in Russia, accounting for 32,2% of all natural foci diseases, that are registered. The incidence rate is 5,7 - 9,7 cases per 100000 people. The highest level of incidence is in the north-eastern part of Siberia.

The incidence rate of TBE takes second place in tick-borne diseases with a decreasing trend for the last 20 years. The average incidence rate is 1,36 - 4,5 cases per 100000 people, with highest levels in Central and Eastern Siberia. During 2005-2014, with a decreasing trend of incidence, in most part of Russian regions 2 or 3 cyclical upturns and recession were registered yet they occurred in different regions during different years.

The cases of “new” tick-borne diseases, such as HME and HGA are also registered every year. For the last 4 years 114 HME and 602 HGA cases were detected. The more localized area of spreading is typical for rickettsiosis (the highest rate is in South of Siberia), CCHF (the southern part of Russia), and ASF (Astrakhan oblast).

P82 Identification of novel Ixodes vaccine candidates using Yeast Surface Display technology

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Ixodes ticks transmit bacterial, protozoal and viral pathogens, causing disease and becoming an increasing health concern in Europe. It is known that repeated tick infestations can lead to 'tick immunity', which leads to reduced tick feeding and partially protects against *Borrelia burgdorferi* infection in laboratory animals. *Ixodes ricinus* and *Ixodes scapularis* are closely related and bioinformatic analysis shows that approximately 54% of *I. ricinus* transcripts have an identity to *I. scapularis* transcripts higher than 80%.

In the current study, a cDNA library constructed of combined salivary gland RNA from nymphal *I. ricinus* ticks feeding for 24, 48 and 72 hours has been cloned into the pYD1 vector and transformed into *S. cerevisiae* EBY-100 cells. Rabbits were repeatedly exposed to *I. scapularis* nymphs feeding to repletion, or *I. ricinus* nymphs feeding for 24 hours, and sera were collected 2 weeks after the last infestation. Purified IgG was used for two screening strategies:1) MACS enrichment and subsequent single cell FACS sorting using tick immune *I. scapularis* rabbit IgG. 2) MACS enrichment with IgG raised against 24h feeding *I. ricinus* nymphs followed by single sorting by flow cytometry using tick immune *I. scapularis* rabbit IgG. Plasmids of isolated single yeast cells were isolated, sequenced and the amino acid sequences were BLASTed against the UNIPROT database. With this approach 13 proteins have been identified that have highly conserved *Ixodes* epitopes and are very likely to be involved in 'tick immunity'. As such, these proteins could be excellent vaccination candidates for a vaccine targeting both *Ixodes ricinus* and *Ixodes scapularis* and might prevent their associated diseases. The potency of the vaccine candidates will be further evaluated using RNAi, as well as vaccination and functional studies.

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P83 Screening for novel I. ricinus vaccine candidates by probing a novel I. ricinus salivary gland Yeast Surface Display with sera from forestry workers

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

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P84 Tick-tattoo: DNA vaccination against tick proteins

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Introduction *Borrelia burgdorferi sensu lato* is the causative agent for Lyme borreliosis. Currently there is no human vaccine against Lyme borreliosis, and most research focuses on recombinant protein vaccines. DNA tattoo vaccination with *B. afzelii* strain PKo OspC (outer-surface protein C) in mice has proven to be fully protective against *B. afzelii* syringe challenge and induces a favorable humoral immunity compared to recombinant protein vaccination.

Method We vaccinated C3H/HeN mice with either *B. burgdorferi sensu stricto* strain N40 OspC using a codon optimized DNA vaccine tattoo based on pVAX1 vector or with recombinant OspC. We subsequently challenged the mice with *B. burgdorferi sensu stricto* strain N40 infected *Ixodes scapularis* nymphs to obtain a valid positive control for infection with *B. burgdorferi sensu stricto* strain N40. Also, we vaccinated 56 C3H/HeN mice with DNA vaccines against five different tick proteins: Salp15, tHRF, TSLPI, Subolesin and Tix-5. These mice were also tick challenged with *B. burgdorferi sensu stricto* strain N40 infected *Ixodes scapularis* nymphs.

Results DNA tattoo and recombinant OspC vaccination induced comparable total IgG responses. The DNA vaccines against Salp15, tHRF, TSLPI, Subolesin and Tix-5 induced IgG responses, albeit lower compared to OspC. There was no significant difference in tick weight and duration of attachment between the different DNA vaccines against tick proteins, compared to the control (or OspC). We intend to measure *Borrelia* loads in mouse skin and deeper tissues by qPCR, determine *Borrelia* growth in skin and bladder cultures, and describe the type of IgG response (IgG1/IgG2) induced by DNA vaccination against tick proteins.

Conclusion If successful, DNA vaccination against tick proteins could be a promising strategy as an easy and feasible vaccination method to provide protection, not only against *Borrelia,* but also against other tick-borne diseases.

P85 Tick-borne encephalitis virus strains of Western subtype isolated in Western and Eastern Siberia of Russia: the genetic and biological properties

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The molecular-genetic analysis of full-genome sequences of 13 European genotype TBEV (TBE-Eu) strains isolated in Western and Eastern Siberia identified two groups of strains corresponded to their isolation areas, differed genetically from each other and had a high level of nucleotide sequences homology within each group. The analysis of polyprotein sequences demonstrated that all investigated TBEV strains regardless of the isolation source differ in the combination of amino acid substitutions at 29 polyprotein positions, distributed in all virus proteins except NS2B protein.

The comparative analysis of the 3'non-coding genome regions sequences of eight TBE-Eu strains from the Western and Eastern Siberia with strains Neudoerfl (U27495) and Hypr (U39292) from Europe was performed. It is found that all studied TBEV strains from Siberia have the deletion in the variable part of 3' non-coding genome region comparable to that one of Hypr strain. The deletion with the maximum length was observed in strains isolated in Western Siberia. It was shown that the deletions in the variable part of the 3'-noncoding genome region were observed both in strain shighly virulent for laboratory mice and in strains with low degree of invasiveness. Such deletions were identified both in the strains isolated from human and the strains from small mammals and ticks.

The evaluation of the virulence degree and hemagglutinating properties of 13 TBEV-Eu strains was performed. It was shown that all studied strains possessed the hemagglutinating ability for goose red blood cells, but had the varying degree of pathogenicity for white nonlinear mice.

It is found that TBEV-Eu strains from Siberia have the high neurovirulence, but some of them, similar to the strains from Europe, exhibits the low invasiveness.

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P86 Bioinformation detection of the structures of recombination sites in genomes of strains of the Siberian subtype of tick-borne encephalitis virus

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One of the leading factors in variability, evolution, adaptation and pathogenesis of viruses is the recombination process. The existence of recombinant events in the tick-borne encephalitis virus (TBEV) is still contested. In this paper, the results of bioinformation search of recombination sites in the genomes of strains of the Siberian subtype TBEV, taken from the GenBank database, are presented. We used software methods for searching for recombination loci from the RDP v 4.61 package (RDP, BootScan, Chimaera, Genecown, MaxChi, SiScan, 3Seq). Only 6 programs (without Bootscan) with a confidence level of $p < 0.5$ registered recombination sites in three strains of TBE: *Tomsk PT-122, Kolarovo 2008, Buzuuchuk*. In the genome of strains *Tomsk PT-122* and *Buzuuchuk*, three recombination sites were identified, and in the *Kolarovo 2008* strain one was found. In the genome of *Tomsk PT-122* strain, the recombination sites were fixed in positions: 7134-8355, 7169-9379, 8356-9379, in the *Kolarovo-2008* strain - 8356-9379, in *Buzuuchuk* strain: 3824-4234, 4580-5106, 5787-6234. Parent strains were identified for the identified recombination sites in the strain Tomsk PT-122: 7134-8355-strains *Kolarovo 2008* and *Vasilchenko*, 7169-9379-*Kolarovo 2008* and *Irkutsk-12*, 8356-9379- *Kolarovo 2008* and *Irkutsk-12.* For the recombinant site in the *Kolarovo-2008* strain, the parent strains were *Tomsk RT-122* and *Irkutsk-12*. Parent strains for recombinant sites in the *Buzuuchuk* strain were: 3824-4234-*Kolarovo 2008* and *Vasilchenko*, 4580-5106-*SibXJX5* and *Vasilchenko*, 5787-6234-*TomskRT-122* and *Vasilchenko*. For the *Tomsk PT-12* strain, the positions of the recombination sites were located in the NS4b and NS5 genes, the *Kolarovo-2008* genome in the NS5 gene, *Buzuuchuk* in the NS2a- NS3 genes. The reported study was funded by the RFBR according to the research project No. 16-04-01336.

P87 Early detection of TBE infection using Reagena vs Virion/Serion TBEV ELISA tests

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Background Infections caused by tick-borne encephalitis virus (TBEV) are very common in north-east Poland. In the early stage of TBEV infection patients present non-specific symptoms what may delay appropriate diagnosis and treatment.

The aim of the study was to check the utility of Rapid TBEV test in comparison with two ELISA assays for the early detection of TBEV infection.

Material/methods The study group included 50 patients (24 women, 26 men) hospitalized (June-October 2016) in the Department of Neuroinfections and Infectious Diseases in Bialystok (Poland) because of TBEV infection. In the control group were 30 patients (15 women, 15 men) hospitalized in summer 2014 because of enterovirus meningitis.

With the Reagena and Virion/Serion kits 50 serum samples and 40 CSF samples were tested for IgG and IgM antibodies. With the Rapid TBEV (Reagena) test 50 serum samples and 20 CSF were tested.

Results Serum samples were IgM positive with 43 (86%) samples by Virion/Serion assay, with 46 (92%) samples by the Reagena assay, and with 47 (94%) samples by the Rapid TBEV test. With CSF samples the Reagena ELISA assay was more sensitive. The IgG results were comparable.

With the control samples all results were negative.

Conclusions The results revealed higher IgM sensitivity of the Rapid TBEV test (Reagena) assay than the Virion/Serion assy in the early stage of TBE.

P88 Long-term persistence of antibody response to tick-borne encephalitis vaccine: 10-year follow-up after first booster dose following different primary vaccination schedules

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Tick-borne encephalitis (TBE) is a viral infectious disease which may cause permanent neurological sequelae or death. Because there is no treatment for TBE, vaccination is the most effective way of prevention and is recommended for individuals who live in or travel to highly endemic areas. Immunogenicity of different primary vaccination schedules followed by a booster dose was previously demonstrated, but data on antibody persistence >5 years post-booster dose is limited.

This phase IV, open-label, second extension study (E2; NCT01562444) assessed antibody persistence ≥6-10 years post-booster dose of TBE vaccine. We present results until year 10.

Subjects aged ≥12 years received 3 primary doses of TBE vaccine according to a licensed (rapid, conventional, accelerated conventional) or modified conventional schedule in the parent trial. Three years post-primary vaccination, subjects were enrolled in an extension study (E1; NCT00387634): 283 received one booster dose of TBE vaccine (40 subjects [rapid group] received it before enrolment in E1) and all (323 subjects) were followed for up to 5 years. Of these, 206 who had received a licenced vaccination schedule were screened to participate in E2. Blood samples were collected yearly; immunogenicity was evaluated by neutralizing antibody (NT) assay.

NT titers peaked 21 days post-booster dose (all groups), then decreased to a plateau without significant differences between primary vaccination schedules. 10 years post-booster dose, NT titer ≥10 was maintained in ≥90% of subjects and geometric mean titers (GMTs) remained high (≥166 across groups); GMTs were lower with increasing age (≥57 [in ≥60-year-olds] versus ≥188 [in 15-49-year-olds]) but remained well above NT≥10 in most subjects (all groups).

Long-term persistence was observed until 10 years post-booster dose of TBE vaccine for all licensed vaccination schedules. This indicates that the 3-5 year interval recommended between the first and a second booster vaccination could be prolonged.

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P89 Comparative analysis of the antigenic properties of the tick-borne encephalitis virus strain used for vaccination

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Tick-borne encephalitis (TBE) is one of the most dangerous natural focal neuroinfections in Eurasia. The study of TBE virus found its spatial genetic separation of genotypes. The basic method of prevention TBE is vaccination and the role of the primary antigen, which is formed by the immune response plays an envelope protein E of TBE. Despite some progress in vaccine-TBE, in the literature there are reports of cases among vaccinated people. One reason for the lack of protection against the virus immunized individuals may be some accumulation of strains in the course of microevolution in a natural conditions and laboratory cultivation of amino acid substitutions that prevent binding of the primary antigen - a protein E with antibodies raised in response to the vaccination.

The objective of this study is to search the GenBank database of the amino acid sequences of E protein of TBE virus associated to the antigenic properties of the virus and comparative analysis of variability in the nature of circulating and vaccine virus strains. The study compared the set of physico-chemical properties of the amino acid residues involved in binding an antibody to an antigen of TBE virus E protein of all strains shown in GenBank database. As a result, it was found that in natural populations circulating strains that differ significantly from the vaccine strains of the physico-chemical properties of amino acid residues in a protein envelope E. This fact points to the need to revise the strategy for developing a vaccine for the prevention of TBE.

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P90 Specific IgM and IgG antibodies against tick-borne encephalitis virus in blood and cerebrospinal fluid

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Background The diagnosis of tick-borne encephalitis (TBE) is based on epidemiological anamnesis, clinical symptoms and laboratory tests. Detection of specific antibodies against TBE virus in blood and cerebrospinal fluid (CSF) mainly confirms the diagnosis. The aim of this study is to present TBE serological findings in blood and CSF of patients from Croatia in a 14-year period. The northwest and northeast continental parts of Croatia between the rivers Sava and Drava and a new natural foci south from the river Sava are endemic TBE regions.

Material/methods From 2002 to 2015 the diagnostic of TBE was made in 9173 blood and 4758 CSF samples with IgM and IgG anti-TBE enzyme immunoassay (EIA) (Sekisui Virotech, Germany). All assays were used according to manufacturer's instructions. The criteria for acute TBE were: IgM high titre, IgM and IgG both positive, and IgG titre minimally four times higher than the cut-off assay value.

Results Anti-TBE antibodies were found in 973 (10.6%) sera and 79 (1.7%) CSF samples. Positive IgM, IgG and both IgM and IgG anti-TBE antibodies were found in 295 (30.3%), 64 (6.6%) and 614 (63.1%) sera and 44 (55.7%), 19 (24.1%) and 16 (20.3%) CSF samples, respectively.

Conclusion The simultaneous detection of IgM and IgG antibodies in blood needs to be performed for TBE diagnosis. When clinical signs appear, patients have mainly developed IgM and IgG antibodies. Specific anti-TBE antibodies in CSF were rarely diagnostically significant. The diagnosis of TBE may not be excluded according to negative CSF antibody findings. Positive IgM anti-TBE antibodies in CSF with concomitant IgM and IgG anti-TBE in serum confirm the acute TBE, but IgM anti-TBE only in serum needs to be obligatory confirmed with IgG testing in consecutive serum.

P91 Sequelae of tick-borne encephalitis in retrospective analysis of 1072 patients

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Tick- borne encephalitis (TBE) is an emerging vector-borne disease in Europe. In spite of the available vaccination many cases continue to be recorded. The aim of the study was evaluation of short and long – term sequelae and analysis of potential risk factors predisposing to sequelae development. Retrospective analysis of medical documentation of 1072 patients was performed.

252 out of 1072 (23.5%) patients developed sequelae. Patients with sequelae were older than patients with no sequelae. Late (> 1 month) subjective sequelae were more frequent than early. Early (< 1 month) neurological sequelae were more frequent than late.

The most frequent early subjective complaints were sleep disorders; late -headache. The most frequent early mental sequelae were concentration disorders; late - memory impairment. The most frequent early neurological sequelae was cerebellar syndrome; late - upper limbs paresis.

Sequelae were observed in 72 patients with meningitis (13.6%), 142 patients with meningoencephalitis (ME) (31.4%) and 38 patients with meningoencephalomyelitis (MEM) (42.2%).

We concluded, that sequelae may affect 23% TBE patients. Neurologic sequelae are the most common group of early sequelae, while subjective symptoms are the most common late sequelae. Patients with MEM are predisposed to neurologic complications, while subjective symptoms were more common in ME. Age and CSF protein concentration were independent risk factors for sequelae development. The risk of late neurologic complications persistence was increased in patients with higher CSF protein concentration. There is a need for better vaccination program, which would greatly prevent the sequelae development.

P92 Assessment of one dose Mannitol influence on hydration and electrolytes concentration in patients with tick-borne encephalitis

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Mannitol administration requires precise monitoring of the patients with neuroinflammatory process in order to prevent water-electrolyte imbalance, because Mannitol treatment may have adverse reactions. The aim of the study was the precise assessment of influence of one dose of 15% Mannitol on patients hydration and electrolytes balance during tick-borne encephalitis (TBE) treatment.

Twenty five patients with TBE were included in the study. Patients were treated with 0.25 g/kg 15% Mannitol per dose. In all patients electrolyte (Na, K, Cl) and creatinine concentrations were measured. First blood sample was taken before first dose of 15% Mannitol. Second blood sample was taken 1 hour after Mannitol administration. Patients hydration status was measured before and 1 hour after Mannitol implementation by whole body bioelectrical impedance with multiple frequency equipment (BodyStat QuadScan 4000).

After Mannitol implementation 7 patients presented with hyponatremia, 3 patients had hypokalemia, 1 patient – hyperkalemia, 15 patients - hypochloremia. One hour after Mannitol implementation the total body water volume changed about 308+-369 ml, internal body water changed about 134+-201 ml and external body water changed about 135+-133 ml. The mean ECW/ICW ratio was 0.7749±0.695 before Mannitol treatment and 0.7736±0.691 one hour after Mannitol treatment. Water and electrolyte changes weren't affected by sex, age and initial hydration status of the patient.

We concluded, that administration of single (0.25 g/kg) dose of Mannitol requires a 300 ml fluid supplementation as well as monitoring for potential hyponatremia. Bioimpedance might be useful in precise monitoring of patients with TBE.

P93 Tick transmission and dissemination of Borrelia crocidurae, relapsing fever agent: analysis on a murine model

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Relapsing fevers (RF) due to *Borrelia crocidurae* represent a major problem of public health in West Africa. In acute infections, *B. crocidurae* can invade the brain. These bacteria are transmitted to human by soft ticks of the genus *Ornithodoros* (*O. sonrai* mainly, *O. erraticu*s in northern Africa) and rodents constitute the main reservoir. Up to now, the physiopathology of soft tick transmission of relapsing fever *Borrelia* to human is not very well investigated.

The aim of this study was to develop an *in vivo* model in Balb/C mouse to better understand the physiopathology of *B. crocidurae* infection. More precisely, we investigated the transmission at the skin interface and *Borrelia* dissemination. The skin was analyzed for: i) *Borrelia* persistence and tissue multiplication, and ii) inflammation and immunity in response to the pathogen and to tick saliva.

Experimentally, *in vivo* reactivation of *B. crocidurae* strain CR24 was made by intraperitoneal injection in mouse. We compared *Borrelia* transmission *via* syringe intradermal inoculation and via *O. erraticus* bite in BalB/C mouse. Skin inflammation and immunity was evaluated by RT-PCR measuring pro-inflammatory cytokines (TNF-α and IL-6), chemokines (MCP-1 and MIP-2) and antimicrobial peptides (cathelicidin-mCRAMP and defensins). Cutaneous multiplication of *Borrelia* was assessed by quantitative PCR of the relapsing fever specific **rrs** gene. Infection follow up was made by blood smears and tissue culture (skin and brain) of *Borrelia* in BSK medium. All the data were collected during a kinetic study of 15 days.

This model, mimicking the natural transmission of relapsing fever, should contribute to a better knowledge of the disease. The role of soft tick saliva in RF *Borrelia* transmission is likely essential as in all well-known arthropods borne diseases and deserves further studies.

P94 Hard ticks (Acari: Ixodidae, Amblyommidae) - potential vectors of Toxoplasma gondii ?

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Toxoplasmosis is zoonosis caused by a protozoan *Toxoplasma gondii.* Consumption of raw or undercooked meat containing parasite cysts is considered as a source of primary infection. Recently, however, some additional routes of transmission are suggested, including infection by transmission by arthropods, especially ticks

The aim of our study was to detect *T. gondi* DNA in ticks and their hosts from different areas of Poland and to estimate their potential role in circulation of the pathogen in the natural environment.

Altogether, 2260 samples were analyzed, including 15 blood and 42 tissue samples from deer, 861 samples of *I. ricinus* collected from the hosts, 512 *I. ricinus* and 830 *D. reticulatus* collected from vegetation in 4 voivodeships: Wielkopolskie (2009), Mazowieckie , Podlaskie (2012-2013) and Pomorskie (2016). Real-time PCR targeting of the B1 gene and a 529 bp repeat element of *T. gondii* was used to detect protozoan DNA, using primers and probes previously described. Plasmids p-JET1with inserted fragment of particular molecular target and DNA isolated from tachyzoites of *T. gondii* served as positive controls. Moreover, a region of the B1 gene was amplified by nested PCR using published primers: Pml/S1 and Pml/AS1 in the first reaction and Pml/S2and Pml/AS2 in the second reaction.

In both real time PCRs all, but control, samples were negative. Moreover, 994 samples were tested by the nested PCR. Of them, three *I. ricinus* from humans, one pool of *I. ricinus* larvae from *Apodemus flavicollis* and two *I. ricinus* from vegetation tested positively. Obtained results show that natural infection of ticks with *T. gondii* is not as frequent as it is suggested by some other data and that a risk of infection in humans due to a tick bite is rather low.

P95 IgE reactivity to α-Gal in relation to Lyme borreliosis

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Background An association between tick bites, the development of immunoglobulin E (IgE) antibodies to galactose-α-1, 3-galactose (α-Gal) and red meat allergy has recently been reported. However, it remains to be shown which tick-bitten individuals that will develop IgE anti-α-Gal, to what extent and the relation to Lyme borreliosis (LB).

Patients and Methods In the highly LB endemic area Kalmar County, Sweden, 518 blood donors with serum samples and health inquiries were included in the study. All sera were investigated for multiple IgG anti-*Borrelia* antibodies using a multiplex bead-based assay (recomBead, Mikrogen). In addition, three serially collected sera over a six month period from 148 patients with clinically defined erythema migrans (EM) were included. IgE antibodies against α-Gal were determined in all sera using ImmunoCAP (Thermo Fisher Scientific).

Results In blood donors reporting previous LB (n=124) IgE to α-Gal was found in 16%, while in donors denying previous LB but with multiple anti-*Borrelia* antibodies (n=94; interpreted as asymptomatic LB) 10% was IgE α-Gal-positive. Finally, in donors without *Borrelia* antibodies denying previous LB (n=300) 14% showed IgE to α-Gal. No significant difference in proportions among groups were found. In EM patients IgE to α-Gal was found in 32/148 (22%) at diagnosis, 31/148 (21%) after two-three months and 23/148 (16%) after six months. A reduction of proportion and level of IgE to α-Gal was found between the second and third sample ($p<0.01$). A positive IgE anti α-Gal was more common among men compared with women both in blood donors and in EM patients ($p\leq0.01$).

Conclusions IgE to α-Gal reactivity showed no significant relation to previous LB. However, IgE anti-α-Gal reactivity in EM patients peaked within 3 months of diagnosis of EM, then the reactivity waned. IgE anti α-Gal was more common in men compared with women.

P96 Co-infection of patients with acute Babesiosis with Lyme disease

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Many publications address the co-infection of patients diagnosed with Lyme disease with babesiosis. In a set of 6,753 samples submitted to our reference laboratory in 2016 form the northeastern US region that were positive for Lyme by our antibody capture EIA and tested for *Babesia microti* infection by PCR, 4.8% were found to be co-infected, which is a rate in agreement with published reports. What is not widely published is the co-infection of patients presenting with acute *Babesia microti* infection with Lyme disease. We analyzed a set of 731 samples that were submitted to our reference lab in 2016 and were positive for *Babesia microti* by PCR and also tested for Lyme infection by antibody capture EIA. The co-infection rate with Lyme disease of patients with acute Babesiosis was found to be 43.9%.

This data and the assays will be further discussed. A discussion of the measure of true co-infection will be included, measured by early stage Lyme infection by antibody capture EIA. Data is also presented on less common co-infection of *Babesia* with *Anaplasma*.

Another measure of co-infection of acute disease can be provided by analysis of our Borrelia species PCR test and our *Babesia microti* PCR test. The *Borrelia* species PCR test employs *Borrelia* species wide primer set, which captures *B. burdorferi* infection but also *B. miyamotoi* infection. In 303 patient samples positive by *Borrelia* species PCR and also tested by *B. microti* PCR, we found a 6.3 % co-infection rate with Babesia when positive for Borrelia.

Exhibitors

Ärztezentrale, Vienna, Austria

Biovendor, Vienna, Austria

Euroimmun, Lübeck, Germany

Oxford Immunotec Ltd., Oxford, UK

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