Introduction

We are proud to present the abstract book of ICLB2022, the 16th edition of the International Conference on Lyme borreliosis and other tick-borne diseases, in Amsterdam, The Netherlands. This edition is co-organized by ESGBOR and NorthTick.

We are grateful for all the abstracts on a great variety of topics ranging from ecology to pathogenesis and from diagnostics to prevention of Lyme borreliosis as well as other tick-borne diseases.

The abstracts are included in the abstract book in an unedited form, i.e. how they were submitted through the online portal. Of note, the abstract book is intended for those who have registered for the meeting only and should not be published, copied, edited or shared with others in any form.

Kind regards,

The conference chairs,

Hein Sprong (WUR) and Joppe Hovius (Amsterdam UMC)
Inhoud

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**Oral presentations**

A novel laboratory approach to discriminate active Lyme borreliosis from non-Lyme individuals in addition to anti-Borrelia serostatus

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**Diagnostics, September 7, 2022, 8:50 AM - 10:30 AM**

**Introduction**
Diagnosis of Lyme borreliosis (LB), except for erythema migrans, relies on patient medical history and examination in combination with laboratory investigations. Indirect laboratory methods, namely serological assays, represent the majority of the methods used in the routine clinic. Unfortunately, the clinical value of *Borrelia* serology results may often be limited. In particular, a persisting anti-*Borrelia* antibody response after a past, not active, exposure constitutes a major limitation, especially in highly endemic geographical areas. The consequence of this is a risk for overdiagnosis and thereby overuse of antibiotics. Additional tests to discriminate an active LB infection from a previous exposure would improve the diagnostic process significantly.

**Aims**
In this study, we wanted to investigate serum samples from clinically well characterised LB patients and healthy controls for a large number of immunological protein biomarkers in order to possibly distinguish patterns typical for active LB from patterns found in previous LB and healthy controls.

**Methods**
Serum samples from well characterised adult patients with ongoing LB together with serum samples from healthy blood donors were included. Serum samples were investigated using a proximity extension assay (provided by Olink®) by which 92 different immune response-related human protein biomarkers were analysed simultaneously.

**Results**
The adult LB cases (n=52) comprised 41 cases of definite Lyme neuroborreliosis, 7 cases of acrodermatitis chronica atrophicans and/or Lyme arthritis and 4 cases of erythema migrans. The healthy controls consisted of 34 blood donors with and 41 blood donors without a positive anti-*Borrelia* antibody result (Enzygnost Borrelia Lyme IgM/IgG). In total, 10 of the investigated 92 immune-related proteins showed a p<0.1 comparing cases with controls, thereby qualifying for further logistic regression. In this analysis, six proteins remained as significantly different between LB patients and controls (p<0.05); PPP1R9B, PRDX5, ITM2A, EIF4GI, DDX58 and ITGB6. These six proteins were then combined in an index for which the discriminating power was determined in a receiver-operating-characteristic curve analysis showing an area under the curve of 0.964 (p<0.001). Age and sex had no impact on the odds ratio of the index.
Conclusion
The results from this study using a powerful multiplex technology show a strong potential possibility to distinguish a present from a past *Borrelia* infection including healthy seronegative controls using a combination of immune-related protein biomarkers. The results now need confirmation in other clinical materials. Although this method is not adapted for routine clinical use at this point, the possibility is interesting and may open new diagnostic opportunities considerably improving the laboratory diagnostics of LB.
A smartphone app to better understand human behavior and tick encounters: The Tick App an evolving tool

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Prevention, September 7, 2022, 11:00 AM - 12:20 PM

Introduction

Human tick encounters depend on the density of infected ticks in the environment (hazard) and on human exposure behaviors such as outdoor activities and tick bite prevention use. While the patterns and drivers of the hazard are fairly well understood, human behaviors linked to exposure, including the effect of tick encounters on the frequency of tick-borne disease preventative actions, have been less studied.

Aims

To better understand human behavior linked to tick encounters, we developed the Tick App smartphone application. Specifically, we aimed to assess 1) similarities and differences in risk factors associated with tick encounters in the United States Northeast and Midwest; 2) drivers and frequency of tick-borne disease prevention method use; and 3) the ability of an in-app intervention, a behavioral change prompt, to improve the frequency of prevention method use.

Methods

The Tick App became available in Google Play and iTunes in spring 2018, when recruitment for participants from New York City, Wisconsin and Michigan began. The app comprises an enrollment questionnaire assessing baseline tick encounter risk; a daily log to characterize outdoor activities and prevention methods used; a tick report to share information about tick encounters, including the ability to upload photos for species identification; educational material and Ixodes scapularis activity levels at the county level are available. Since 2020, participants are randomly assigned to a control or treatment group to receive a message on the app’s opening screen reminding them to do a tick check (control) or to do a tick check or one of two other prevention strategies that should be used more frequently (treatment). These messages use behavior change techniques, goal striving, motivation enhancing, and planning and preparation.

Results

A total of 14,955 participants completed the enrollment survey from 2018 to 2021. They completed 30,444 daily logs and 11,439 tick reports. Participants in the two regions had different environmental risk factors and activity patterns: for example, Midwest participants conducted outdoor activities more frequently. During COVID-19 stay-at-home-orders peridomestic outdoor activities and tick encounters were more common compared to 2019. Checking for ticks was the most commonly reported tick bite prevention strategy (~80%); however, few participants checked daily for ticks after conducting outdoor activities. Participants who were prompted to shower and wear bug spray were not more likely to use these strategies compared to the control group. Interestingly, the control group had a higher tick check frequency.
Conclusion
The Tick App provides a valuable way to connect with local communities and to collect a wealth of data. This work serves as a starting point to further develop smartphone-based applications to improve tick-borne disease prevention and to further dissect the intricate relationship between human behavior and environmental tick hazard.
Abundance in the host and tick is critical for the transmission success of *Borrelia burgdorferi* sensu lato

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Ecology, September 6, 2022, 11:00 AM - 11:30 PM

*Borrelia burgdorferi* sensu lato is a complex of tick-borne spirochetes that include the causative agents of Lyme borreliosis. *B. burgdorferi* is maintained in nature by enzootic cycles that include hard ticks in the genus *Ixodes* and vertebrate reservoir hosts. The life cycle of *B. burgdorferi* includes two critical steps: (1) transmission from an infected host to naïve larvae and (2) transmission from an infected nymph to a naïve host. Host-to-tick transmission (HTT) can be measured by infesting infected hosts with larval ticks and determining the percentage of ticks that acquire the infection. Tick-to-host transmission (THT) is inherently more difficult to quantify. *B. burgdorferi* has a high reproduction number (R0) compared to other tick-borne pathogens because it establishes a chronic infection in the reservoir host with high HTT.

In endemic areas, *B. burgdorferi* populations often consist of a dozen antigenically distinct strains that circulate in the same vertebrate host and tick populations. Comparative studies with multiple strains allow us to better understand how natural selection shapes the life history traits of *B. burgdorferi*. Our studies have found that strains that establish higher abundance in the tissues of the vertebrate reservoir host have higher transmission to feeding ticks. Similarly, strains that establish a higher abundance in the nymphs have a higher probability of being transmitted to naïve reservoir hosts.

In nature, co-infections with multiple strains of the same *B. burgdorferi* species are common in both tick vectors and vertebrate reservoir hosts. Co-infection results in competitive interactions where the presence of one strain reduces the fitness of the other strain and vice versa. Co-infections in the vertebrate host reduce the abundance of strains in the host tissues, which in turn reduces the strain-specific probability of HTT. The strain that wins the competition for transmission to ticks is the one that establishes the higher abundance in the tissues of the co-infected rodent host, but whether this result is general remains unknown. In summary, the ability to maintain high abundance in the host tissues and the nymphal tick is critical for strains to have high transmission success in both steps of the tick-borne life cycle.
Antibiotic treatment length and clinical features in European Neuroborreliosis: Two versus six weeks treatment with doxycycline

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Lyme in the Lymelight, September 6, 2022, 4:20 PM - 5:30 PM

Introduction
Current guidelines recommend antibiotic treatment for 14 to 21 days in European neuroborreliosis. There is, however, limited evidence regarding optimal duration, and clinical practice varies. Studies report persisting symptoms for some patients, regardless of treatment strategies. This study aimed to compare efficacy and safety of oral doxycycline for two and six weeks. We also wanted to investigate clinical and inflammatory features, persisting complaints and prognostic factors.

Methods
The trial had a randomised, double-blinded, placebo-controlled, non-inferiority design. One hundred and twenty-one patients with neuroborreliosis from eight Norwegian hospitals were randomised to doxycycline 200 mg daily for two or six weeks. The primary endpoint was clinical improvement as measured by difference on a composite clinical score from baseline to six months. Secondary endpoints were composite clinical scores at ten weeks and 12 months, patient reported questionnaires, lab data and side effects. The non-inferiority margin was predetermined to 0.5 points.

Results
Out of 121 included patients, 52 were treated for two weeks and 53 for six weeks, and included in the intention to treat principle analyses. 52 and 51 were included in per protocol analysis. Ninety-two patients were followed for 12 months after inclusion. Mean difference in clinical improvement at six months between the groups was 0.06, 95% CI -1.2 - 1.2, p = 0.99. Neither inferiority nor non-inferiority could be established. The groups were also similar in terms of secondary endpoints. There were no treatment failures, nor serious adverse events.

Conclusion
Even if non-inferiority could not be established, our study did not show benefits of extending antibiotic treatment beyond two weeks. Further long-term data on outcome and possible prognostic factors will be presented.
Are generalists species replacing specialists? Implications of hosts species distribution on tick-borne diseases along an altitudinal gradient in the Italian Alps.

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Introduction
In a global change context, the association between generalists and specialists could be a determinant of ecosystem stability. This holds particularly true in mountain ecosystems, where the environmental tolerance of generalists to global warming may lead to upward distributional shifts and thus declining of specialists. As a consequence of this altitudinal expansion, even the parasites that these species carry can shift, eventually promoting the emergence of infectious diseases in newly colonized areas. We examined these relationships in small mammals along an altitudinal gradient of Alpine habitats, analysing the differences in small mammals’ assemblage and pathogens’ occurrence.

Methods
We capture-mark-recaptured wild small mammals from 500 to 2500 m a.s.l. at 500-meters intervals in the Italian Alps, in 2019 and 2020. We counted ticks on rodents and collected ear biopsy samples. Molecular PCR-based methods coupled with sequencing and serological assays were performed for vector-borne pathogens screening. We analysed small mammal species assemblages and probability of infection along the altitudinal gradient with Redundancy Detrended Analysis (RDA) and Generalized Linear Mixed Models (GLMMs).

Results
In total we captured 333 animals belonging to 11 species (Apodemus flavicollis, A. sylvaticus, Chionomys nivalis, Microtus arvalis, M. subterraneus, M. agrestis, Myodes glareolus, Sorex araneus, S. alpinus, S. minutus, Crocidura leucodon) and counted 3782 ticks (3718 larvae and 64 nymphs) belonging to the genus Ixodes. A. flavicollis and My. glareolus occupied in sympathy the montane belts, from 500 to 1500 m a.s.l. My. glareolus was also present in the alpine belts, from 2000 to 2500 m, together with C. nivalis, Microtus spp. and shrews. We also detected an unevenly altitudinal pattern of distributions of vector-borne pathogens, with Borrelia spp. occurring up until 1500 m a.s.l. (prevalence 13.88%), while Anaplasma phagocytophilum (7.09%) and Babesia microti (3.08%) mainly recorded in alpine belts.

Conclusions
Wild rodents are important reservoirs of numerous tick-borne pathogens. The altitudinal segregation that we found, revealed that also parasites are affected by climate, as shown by the presence of A. phagocytophilum and B. microti at higher altitudes where I. ricinus has never been recorded. This made us speculate on the presence of a more specialist endophilic tick species that solely utilize small mammals as hosts for all developmental stages (such as Ixodes trianguliceps). Moreover, our findings support the expansion of the generalist species My. glareolus toward higher altitudes, where specialists, such as C. nivalis, were restricted only in
some specific habitats. The potential replacement of specialists by generalists can cause an homogenization at the community level which in turn could alter ecosystem functioning, including the host-parasite-pathogen association with implication for emerging infectious diseases spread.
Autoimmunity to synovial fibroblast-derived extracellular matrix proteins in patients with post-infectious Lyme arthritis

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New and Hot, September 5, 2022, 8:50 AM - 10:20 AM

Introduction+Aims
Fibroblast-like synoviocytes (FLS) are critically important cells in post-infectious Lyme arthritis (LA) and in other chronic inflammatory arthritides. In inflamed tissue, FLS upregulate cell-surface expression of antigen-presenting molecules, especially HLA-DR molecules. However, little is known about the antigens presented by these cells. To better understand disease pathogenesis, we identified T cell epitopes of FLS-derived extracellular matrix (ECM) proteins, determined Borreliella (Borrelia) burgdorferi (Bb)-mimic epitopes, and characterized T and B cell responses to these peptides or proteins.

Methods
HLA-DR-presented peptides (T cell epitopes) of FLS-derived ECM proteins were identified directly from synovia of post-infectious LA patients using mass spectrometry. T and B cell responses to these proteins were determined by ELISpot and ELISA assays. Bb-mimic epitopes were identified by BLAST searches and tested for immunogenicity. Tetramer reagents were used to test directly the binding of these epitopes to patients’ CD4+ T cells and to determine the subtypes of these cells.

Results
Using immunopeptidomics to examine LA patients’ synovial tissue, we identified 3 HLA-DR-presented peptides (T cell epitopes) of fibroblast-derived ECM proteins - fibronectin-1, laminin B2, and collagen Va1. When tested for immunogenicity, 14 of 24 patients (58%) with post-infectious LA had CD4+ T cell responses to ≥1 of these T cell epitopes, and 9 of 52 (17%) had antibody responses to ≥1 of these proteins. These responses were found almost exclusively in patients with post-infectious LA, the period when massive FLS proliferation develops. Post-infectious LA patients with these T cell responses had significantly increased frequencies of HLA-DRB1*04 or DRB1*1501 alleles compared with antibiotic-responsive LA patients (P=0.0003), and among post-infectious LA patients, the duration of arthritis in the post-infectious period was significantly longer among those who had responses to FLS-derived protein epitopes compared with those who lacked these responses (P=0.05). Of the 8 patients who had T cell reactivity with the collagen Va1\textsuperscript{1730-1750} epitope, 5 also had responses to a similar Bb-mimic epitope in a previously unrecognized BBQ62-like protein, which is encoded on lp28-2. Tetramer reagents bound the autoreactive or Bb-mimic epitope, and a small percentage of cells bound both the borrelial and collagen epitopes. A high percentage of autoreactive CD4+ T cells to this one collagen epitope were T-bet-positive Th1 cells, intermediate percentages were GATA-2-positive Th2 cells or RoR\gammat-positive Th17 cells, and only a small percentage were FoxP3-expressing Treg cells.
Conclusion
These findings offer new insights into the pathogenesis of post-infectious LA. We propose a hypothesis linking 1) infection of collagen in joints with markedly inflammatory *Bb* strains, 2) induction of hyperinflammatory responses and marked proliferation of FLS, and 3) molecular mimicry between borrelial and collagen epitopes leading to collagen-reactive CD4+ T cells, which likely play a role, in part, in prolonging joint inflammation in post-infectious LA.
**Borrelia burgdorferi** PlzA is a c-di-GMP dependent DNA binding protein

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New and Hot, September 5, 2022, 8:50 AM - 10:20 AM

Introduction

Cyclic-di-GMP (c-di-GMP) is a ubiquitous secondary signaling molecule utilized by many bacteria to mediate regulation in response to extracellular stimuli. Bacteria that utilize c-di-GMP possess proteins that bind c-di-GMP and mediate various effector functions. The Lyme disease spirochete, **Borrelia burgdorferi**, has a single c-di-GMP binding protein, PlzA, but the mechanism of function of PlzA was not previously known. We have determined that PlzA is a DNA binding protein and that DNA binding function is c-di-GMP dependent. Given the singularity of PlzA to *B. burgdorferi*, it could be a novel target for therapeutics against Lyme disease.

Methods

Recombinant wild-type PlzA, mutant PlzA (cannot bind c-di-GMP), and the N and C terminal domains of PlzA were generated through molecular cloning and expressed in *E. coli*. Electrophoretic mobility shift assays (EMSA) were used to assess the DNA binding function and specificity of the recombinant proteins to fluorescently labeled probes derived from DNA sequences of the B31 strain of *B. burgdorferi*. EMSAs were performed with and without the addition of c-di-GMP to determine the effect of c-di-GMP on PlzA DNA binding function.

Results

Wild-type PlzA binds DNA in a c-di-GMP dependent manner, while the mutant PlzA is unable to bind the tested DNAs. Increasing concentrations of c-di-GMP in EMSAs with wild-type PlzA led to increased affinity for DNA. PlzA binds to DNA adjacent to the promoter for glycerol metabolism genes, but not to other *Borrelia* derived sequences tested, indicating that PlzA-binding is site-specific. Dissection of the protein domains of PlzA revealed the N-terminal domain to be the DNA binding domain which can bind DNA independently of c-di-GMP. The C-terminal domain, which contains the c-di-GMP binding sites, does not bind DNA but instead confers DNA binding specificity as the N-terminal domain binds DNA sequences full-length PlzA does not bind.

Conclusion

Our study assigns a mechanism for PlzA effector function. PlzA is a c-di-GMP dependent DNA binding protein. The N-terminal domain was identified as the DNA binding domain whose function is independent of c-di-GMP but lacks DNA binding specificity. The C-terminal domain and c-di-GMP are therefore indispensable for the DNA binding specificity and function of PlzA. Ongoing studies include examining the effects of PlzA on *B. burgdorferi* transcription and translation, identifying potential binding partners and additional DNA targets, mapping the DNA binding sites, and performing site-directed mutagenesis on the PlzA protein to identify amino acid residues required for DNA-binding and other functions.
Changes in the human skin immune landscape facilitate tick-borne pathogen transmission

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Young & Wild: early career researchers’ presentations, September 5, 2022, 4:30 PM - 5:40 PM

Introduction:
During tick feeding, the skin becomes a site of transmission for immunomodulatory salivary compounds, which are thought to facilitate human infection with tick-borne pathogens including Borrelia burgdorferi sensu lato species. However, due to lack of model systems, precise cellular changes in the human skin immune cell network upon tick feeding remain to be investigated.

Methods:
We assessed human skin samples taken at the sites of tick bites (n=19) compared to matched intraindividual control skin (n=19) and performed multicolor flow cytometry, cytokine stimulation assays, tissue immunofluorescence and PCR for tick-borne pathogens. Cellular changes detected in tick bite skin biopsies were replicated in a human skin explant model using Ixodes ricinus tick salivary gland extracts. Lastly, we modeled infection with tick-borne pathogens ex vivo, using subepidermal injection of B. burgdorferi sensu stricto and B. afzelii spirochetes into human skin explants.

Results:
Within hours following the bite, neutrophils, B and T cell populations increased, suggesting an initial inflammatory response independent from pathogen transmission. While a higher number of cutaneous T cells expressed tissue-residency markers, cytokine production of T cells and innate lymphoid cells (ILC) was impaired. Notably, tick bites elicited weak systemic effects on peripheral blood lymphocyte populations. Corresponding to our results of tick bite lesions, we observed an increase in total T cell numbers and tissue-resident memory T cells after tick salivary gland extract injection to human skin explants. Subepidermal injections of B. burgdorferi and B. afzelii strains resulted in bacterial repilaction within the skin explant, with differential dynamics of the two strains. In early stages of model infections, macrophages and dermal DC were in close spatial relationship to spirochetes, indicating pathogen uptake. Interestingly, pre-incubation of spirochetes with tick salivary gland extracts hampered accumulation of immune cells and increased spirochete load ex vivo.

Conclusions:
Collectively, we show that tick feeding exerts profound changes on the skin immune network, which interfere with the primary response against tick-borne pathogens. This detailed map of
cell-specific changes and leukocyte-pathogen interaction may allow designing effective prevention and treatment strategies for tick-borne diseases in the future.
Characterization of the protective antibody response in babesiosis by use of whole pathogen proteome array and a mouse model of cd4 deficiency

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Pathogenesis, September 6, 2022, 8:50 AM - 10:20 AM

Introduction
Immunocompromised patients are at risk of severe babesiosis. Those for whom the humoral response is impaired due to ongoing or recent treatment with B-cell depleting agents are at risk of severe, relapsing babesiosis. Thus, in the context of relative immune suppression, antibodies appear critical for an effective host response to B. microti.

Aims
The protective antibody response to B. microti remains uncharacterized. We previously established that B cells and class switch recombination are critical for resolution of severe B. microti infection in cd4-deficient mice. The aims of this study were: i) to understand how cd4-deficient mice mount an effective antibody response to B. microti, and ii) to characterize the antigen specificity of this antibody response.

Methods
Wild-type and cd4-deficient mice were infected with B. microti. Parasitemia was monitored by use of flow cytometry. Plasma was obtained during resolution of infection and probed for IgG reactivity to B. microti antigens using arrays spotted with full-length proteins or partial but overlapping peptides that encompass 91% of the expressed B. microti genome. Splenic immune cells were localized by immunofluorescence. Frequencies of cytokine-expressing T cells were determined by intracellular staining. Mice that lack a cytokine receptor of interest were generated and infected with B. microti.

Results
At time of full resolution of infection, we identified 62 IgG reactive proteins when probing plasma from wild-type mice but 43 when probing plasma from cd4-deficient mice. Of the 43 proteins, 15 were already reactive at time of peak infection and 19 became reactive at time of partial resolution. For three of the 34 antigens, IgG titers and parasitemia were inversely correlated at time of partial resolution. One was already reactive at the time of peak infection whereas the other two gained reactivity as the infection resolved. Four additional antigens were predicted to traffick through the endoplasmic reticulum, thereby increasing the odds of antibody-mediated neutralization. All seven antigens have little to no genetic variation in their coding region. In agreement with the role of IL-21 in germinal center formation, resolution of infection in cd4-deficient mice required the IL-21 receptor but not the IFN-gamma receptor. Populations of CD8+ T cells and CD4 CD8- T cells expanded as the infection unfolded. Unlike CD8+ T cells, CD4 CD8- T cells were found at the germinal centers. CD4 CD8- T cells expressed IL-21 and IFN-gamma. When compared with CD4+ T cells of wild-type mice, IL-21 expression by CD4 CD8- T
cells was delayed but IFN-gamma expression was not. Importantly, primary infection of $cd4$-deficient mice protected from reinfection.

**Conclusion**
We have identified seven promising antigens for the development of a subunit vaccine against babesiosis. We also have established the mouse model of $cd4$ deficiency as a powerful tool to identify and test vaccine candidates.
**Chromosome segregation in polyploid *Borrelia burgdorferi* spirochetes is orchestrated by a novel centromere-binding protein, ParZ**

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Young & Wild: early career researchers’ presentations, September 5, 2022, 4:30 PM - 5:40 PM

**Introduction**

Cellular replication is essential for the transmission of the Lyme disease pathogen *Borrelia burgdorferi* and for its ability to infect its vertebrate host. As part of this process, faithful genome replication and segregation ensures that each daughter cell inherits one complete genome copy. Comprising of one chromosome and about 20 plasmids, many of which are required *in vivo*, *B. burgdorferi*’s genome is the most segmented among known bacteria. Its cells are thought to contain one or two copies of the genome, a property which has widely been used to determine tissue loads by polymerase chain reaction. It is not known how this unusually segmented genome is organized within the spirochete cells and how the two copies of the replicated genome are segregated across tens of microns of cellular space.

**Methods**

We used extensive genetic manipulations, fluorescent protein tagging, and fluorescence imaging to specifically and for the first time label multiple genome loci in the *B. burgdorferi* type strain B31 and several other strains. Using quantitative image analysis, we characterized the subcellular localization of the genome segments and quantified the involvement of the Par proteins in *B. burgdorferi* chromosome segregation. We confirmed our findings using whole genome sequencing and chromatin immunoprecipitation-sequencing.

**Results**

We determined the subcellular localization of multiple chromosomal loci and of 16 endogenous *B. burgdorferi* plasmids. To our surprise, exponentially growing *B. burgdorferi* cells were polyploid, with about 9 chromosome copies per cell. Importantly, *B. burgdorferi* cells were also polyploid inside the tick vector, rendering the polyploidy finding physiologically relevant. We also show that the chromosome and plasmids are regularly spaced along the length of the spirochetes. We therefore investigated the control of regular chromosome spacing by the Par proteins. In *B. burgdorferi*, we found that the centromere-binding protein ParB lost its ability to control the ParA ATPase, diminishing its participation in chromosome segregation. We show that *B. burgdorferi* instead has a previously uncharacterized and likely phage-derived centromere-binding protein, which we dubbed ParZ. ParZ recognizes a novel centromere site located within its own coding sequence and controls ParA localization. We showed that ParA and ParZ have a major role in chromosome segregation.
Conclusion

*B. burgdorferi* polyploidy and regular distribution of its genomes along the length of the cell ensure faithful genome inheritance during symmetric division. We showed that regular chromosome spacing is controlled by a novel centromere-binding protein. Additionally, our polyploidy finding has implications for bacterial tissue load determination and assessment of pathogenic potential and response to treatment.
Development and validation of a protein array for Lyme borreliosis diagnostics

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Diagnostics, September 7, 2022, 8:50 AM - 10:30 AM

Introduction
For Lyme borreliosis (LB) diagnostics two-tier testing is recommended, which usually consists of a screening ELISA and confirmatory immunoblot. This is laborious, prone to inter-observer variability and expensive.

Aim
We aimed to develop and validate a one-tier protein microarray for LD diagnostics using multiple Borrelia burgdorferi sensu lato antigens, which are currently used in commercially available serological tests.

Methods
Specific IgM and IgG antibodies were detected using a microarray containing 12 Borrelia afzelii and Borrelia bovarensis (Bbsl) antigens. Serum samples of culture-confirmed LB patients were used for validation, consisting of 58 patients with erythema migrans (EM, 19 acute, 20 early and 57 late time-point samples), and 13 patients with disseminated LB in combination with a skin manifestation (2 acute, 4 early, 20 late time-point samples). As endemic and non-endemic healthy controls, samples of 97 individuals in Izhevsk, Russia, and 83 individuals in Volgograd, Russia, were used respectively. As a potentially cross-reactive cohort, 283 sera samples of 83 patients with PCR-proven Borrelia miyamotoi disease (BMD) were used. The cut-off for reactivity for each individual antigen was set at 5 μg/ml for IgM and IgG based on ROC-analyses, and an interpretation algorithm was developed by stepwise logistic regression.

Results
IgM-positivity was defined as reactivity against OspC or two of the following proteins; VlsE, p58, p39, p41 or BBK32. IgG-positivity was defined as reactivity against VlsE or two of the following proteins; p100, p58, p41, p39, BBK32 or OspC. A sensitivity of 48% (95% CI 36-70) was observed in acute samples of EM patients, and 95% (95% CI 92-97) in late samples of disseminated LB for IgM/IgG. Regarding the EM cohort, the overall protein microarray sensitivity was 66% (CI 95% 54-78), and concerning the disseminated LB cohort 92% (CI 95% 77-100) for IgM/IgG. Specificity was 96% (CI 95% 92-100) for IgM/IgG in the healthy controls. Furthermore, the protein microarray distinguished BMD from LB patients, with a specificity of 98% (CI 95% 95-100) for IgM, and 97% (CI 95% 93-100) for IgG. We are currently evaluating the sensitivity and specificity in LB patient from the Dutch LymeProspect (n=1125) cohort, Dutch endemic healthy controls (n=600), Norwegian non-endemic healthy controls (n=100), and Russian and Dutch infectious disease controls (n=93 and n=51, respectively).
Conclusion
We have developed and validated a novel protein microarray for LB diagnostics with an appropriate sensitivity and excellent specificity. We are currently evaluating the protein array in additional European cohorts. This novel protein microarray might be a valuable contribution to routine LB diagnostics.
DEVELOPMENT OF A MULTIVALENT LYME BORRELIOSIS VACCINE CANDIDATE: RESULTS FROM A PHASE 2 SAFETY & IMMUNOGENICITY STUDY IN AN ADULT AND PEDIATRIC STUDY POPULATION

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Prevention, September 7, 2022, 11:00 AM - 12:20 PM

Introduction
Lyme borreliosis (LB) is the most common tick-borne disease across the northern hemisphere. It is caused by several genospecies of *Borrelia burgdorferi* sensu lato spirochetes, which are transmitted to humans via the bite of an infected *Ixodes* tick during a blood meal. Steadily increasing numbers of LB cases and the lack of effective preventive measures emphasize the need for a vaccine. Currently there is no vaccine available for humans. Outer surface protein A (OspA) is a proven target for a LB vaccine. We developed a subunit vaccine candidate (VLA15) which is based on OspA and targets the majority of *Borrelia* strains expressing clinically relevant OspA serotypes (STs) present in Europe (ST1-ST6) and North America (ST1).

Methods
We conducted a randomized, observer-blinded, multi-centre Phase 2 study to investigate the safety and immunogenicity of VLA15 in adult and pediatric study populations. A total of 625 participants aged 5-65 years were enrolled in three age groups (18-65 years, 12-17 years and 5-11 years) and were randomized 1:1:1 to receive VLA15 180 µg w/ alum in a three-dose schedule (Month 0-2-6) or a two-dose schedule (Month 0-6) or three injections of placebo (Month 0-2-6). To date, safety and immunogenicity data up to one month after completion of the vaccination series (Month 7) are available and will be presented.

Results
VLA15 was generally safe and well tolerated in all age groups and in both vaccination schedules tested. The observed safety profile was consistent with licensed lipidated subunit vaccines. The majority of adverse reactions were mild or moderate. No related SAEs or other safety concerns have been identified. VLA15 was immunogenic and induced high antibody levels against all six OspA serotypes in all age groups and with both vaccination schedules tested. Immune responses in the pediatric study groups were significantly higher than in the adults. Overall, a three-dose vaccination schedule induced a more robust immune response than a two-dose schedule. Participants in the ongoing study will be followed-up until one year after completion of the primary vaccination series. At this point in time, participants will receive an additional dose of VLA15 or placebo at Month 18 in order to investigate the effect of a booster dose.

Conclusion
VLA15 was safe and immunogenic in all age groups and schedules tested. A three dose schedule with a primary vaccination schedule of Month 0-2-6 was selected for the upcoming Phase 3 study,
which will investigate the efficacy of the vaccine candidate in a study population 5 years of age and older.
Development of a new diagnostic test for Lyme borreliosis based on antigens discovered by screening a whole proteome microarray

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Introduction
Lyme borreliosis (LB), the most debilitating vector-borne illness in the Northern hemisphere, is caused by Borrelia burgdorferi sensu lato genospecies transmitted by Ixodes ticks. LB is a multi-system disorder with an array of clinical manifestations starting from early skin infection (erythema migrans; EM) as well as disseminated infection (Lyme arthritis, Lyme neuroborreliosis and Lyme carditis) and late skin infection (acrodematitis chronica atrophicans; ACA). LB diagnosis is primarily based on the clinical manifestation (EM rash) and serological testing of Borrelia specific antibodies. However, most recommended tests suffer from two major pitfalls: i) low sensitivity to diagnose early disease and ii) inability to discriminate an active and past infection.

Aim
Development of a new diagnostic test for LB with a high sensitivity in early disease and potential to discriminate an ongoing versus past B. afzelii infection.

Methods
A whole proteome microarray with ~1300 Borrelia afzelii proteins was probed with human serum samples (healthy blood donors (HBDs) n=50 and LB patients at different stages n= 99) and experimentally infected murine samples (controls n= 16 and different infection stages n=16) (discovery set). A validation set, with independent human serum samples (HBDs n=75 and EM n=60), was probed against 3 recombinantly produced antigens for sensitivity and specificity analysis compared to well-established C6 peptide ELISA. Cut-offs were determined by ROC curve and 95% confidence interval (CI) were calculated using Wilson/Brown method with GraphPad Prism 9.1. McNemar’s test was used to calculate statistical significance for sensitivity and specificity.

Results
We identified 202 proteins present in both the human and murine dataset, discriminating HBDs from LB. Antigen1, 2 and 3 demonstrated significantly higher IgG responses at early stage and were screened with ELISAs with an independent validation sample set and compared to C6 peptide ELISA (benchmark). C6 peptide detected early LB with a sensitivity of 79.1% (95% CI 67.2-87.7) and a specificity of 92% (95% CI 83.6.8-93.2). Antigen1 and 2 demonstrated significantly higher sensitivity of 98% (95% CI 91.1-99.1; p=0.001) and 93.3% (95% CI 84-97.3; p=0.03) and a specificity of 96% (95% CI 88.8-98.9; p=0.10) and 94.6% (95% CI 87-97.3; p=0.31), respectively. Antigen3 demonstrated sensitivity of 81.6% (95% CI 70-89.4; p=0.61) and specificity of 93.3% (95% CI 85.3-
97.1; p=0.73) comparable to C6 peptide. Antigen3+C6 peptide demonstrated a significantly high sensitivity of 91% (95% CI 80.7-96.1; p=0.008) and comparable specificity of 92% (95% CI 83.6-96.2; p=0.15) to C6 peptide alone.

**Conclusion**

We report three novel diagnostic proteins demonstrating high sensitivity (2/3 highly sensitive than C6 peptide) and specificity in early LB as a single-tier test. We are currently evaluating their IgM responses, their potential as discriminator of active and past disease and planning a larger scale evaluation of proteins for development of a clinical diagnostic test for LB.
Diagnostic parameters of cellular tests for Lyme borreliosis in Europe (VICTORY study): a case-control study

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Lyme in the Lymelight, September 6, 2022, 4:20 PM - 5:30 PM

Background
Cellular tests for Lyme borreliosis (LB) may be able to overcome major shortcomings of serological testing, such as its limited sensitivity in early LB. We therefore assessed the sensitivity and specificity of these cellular tests.

Methods
This is a nationwide prospective case-control study performed in the Netherlands. Cases were consecutively included patients with physician-confirmed LB at the start of antibiotic treatment (n=271, of whom 245 with early localized LB). Controls comprised 228 healthy persons from the general population and 41 persons with potentially cross-reactive conditions. We performed three cellular tests for LB (Spirofind Revised, iSpot Lyme, LTT-MELISA) as index tests, and standard two-tier serological testing (STTT) as comparator.

Findings
The specificity in healthy controls of STTT (216/228, 94·7%, 95%CI: 91·5-97·7) was higher than that of the cellular tests: Spirofind (140/171, 81·9%, 95%CI: 76·1-87·2), iSpot Lyme (32/103, 31·1%, 95%CI: 21·5-40·3) and LTT (100/190, 52·6%, 95%CI: 44·9-60·3). Cellular tests had varying sensitivities: Spirofind (88/204, 43·1%, 95%CI: 36·4-50·4), iSpot Lyme (51/94, 54·3%, 95%CI: 44·5-63·7) and LTT (66/218, 30·3%, 95%CI: 23·8-36·7). The Spirofind and iSpot Lyme outperformed STTT in this respect, while having comparable sensitivity as the C6-ELISA (C6-ELISA: 135/270, 50·0%, 95%CI: 44·5-55·5; STTT: 76/270, 28·1%, 95%CI: 23·0-33·6).

Interpretation
The cellular tests for LB under study have a low specificity in comparison to serological tests, which leads to a high number of false-positive test results. We conclude that they are unfit for clinical use at this stage. This is especially relevant for the iSpot and LTT, which have been used for patients for years.

Funding
Netherlands Organization for Health Research and Development, AMC Foundation, Ministry of Health of the Netherlands

Keywords
Lyme disease, borreliosis, erythema migrans, Borrelia, diagnostic accuracy, cellular tests, serology
Diet-derived compounds modulate the innate immune response to

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Pathogenesis, September 6, 2022, 8:50 AM - 10:20 AM

The emergence of the concept of trained immunity a decade ago has redefined our comprehension about the immune system both during steady-state and disease. Indeed, several contributions have characterized the transcriptomic and metabolic reprogramming induced in innate immune cells by their previous exposure to single PAMPs or vaccine formulations, which in turn, renders these cells hyper or hypo responsive to subsequent encounters with inflammatory insults. Our group has described the memory-like responses in macrophages upon their prior exposure to live B. burgdorferi, and how this memory phenotype is amenable to be manipulated in vivo to reduce the cardiac inflammatory output during murine Lyme borreliosis. A growing body of evidence has shown that this trained phenotype is also imprinted into hematopoietic stem cells (HSCs, known as central trained immunity), resulting in a pre-conditioned mature functional cell that displays differential responses to diverse stimuli. Moreover, the capacity to induce trained immunity has been related to the activity exerted by several molecules including microbiota-derived metabolites, establishing new opportunities to identify putative modulators of the immune system.

We have characterized the functional response of murine bone marrow macrophages (BMM) pre-activated with phenolic compounds derived from the diet and re-stimulated with LPS or different bacterial species, including B. burgdorferi. Furthermore, we have assessed the inflammatory potential of stem cells differentiated into BMM in the presence of these phenolic compounds. Further, we have studied the long-lasting effects induced by these metabolites on HSCs in vivo by pre-treating mice one month before challenge with B. burgdorferi. Our results show a significant decrease in the release of inflammatory factors in BMM previously activated with phenolic compounds and challenged with different bacteria. Indeed, a reduction in TNF production upon stimulation was also observed in HSCs differentiated into BMM in the presence of these metabolites. Furthermore, this modulatory effect is maintained over the long-term after treatment. In fact, animals administered products of microbial gallotannin metabolism one month prior to infection with B. burgdorferi showed reduced cardiac macrophage infiltration and inflammation.
Our results show that the generation of central trained immunity has profound consequences in the generation of proinflammatory responses in response to infection with the spirochetal pathogen.
Epidemiology and costs of Lyme borreliosis infections in Germany – a retrospective claims data analysis

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**Introduction**

Lyme borreliosis (LB) is an infectious, vector-borne disease caused by the spirochete *Borrelia burgdorferi sensu lato* and mainly transmitted by Ixodes ticks. With public surveillance established in only 9 out of 16 federal states, the epidemiology and healthcare burden of different LB manifestations like erythema migrans (LB/EM), Lyme neuroborreliosis (LNB) and Lyme arthritis (LA) in Germany is relatively unknown.

**Methods**

Using the WIG2 benchmark database, we analyzed health claims data from 3.044 to 3.321 million insured statutory health insurance (SHI) fund members in 2015 to 2019. With specifically designed case definitions for each manifestation, including a combination of ICD-10 codes, antibiotic prescriptions, and/or laboratory test orders, we calculated LB incidence (LB/EM and disseminated LB) per 100,000 population, both nationally and by federal state. Incidence and KM 6-statistics were used to extrapolate cases to the entire SHI population (73,009,237 in 2019). Using 1:4 propensity-score matching (by age, sex, comorbidities and region), we composed a matched control population to compare LB-induced SHI costs, healthcare resource utilization (HCRU) and mortality over three-years following an incident LB diagnosis in 2015 and 2016.

**Results**

In 2019, LB/EM incidence was 241/100,000 population in Germany. LNB and LA incidence was 9.5 and 6.2 per 100,000, respectively. LB/EM incidence was highest in Saarland and Saxony (495 and 476/100,000, respectively), and lowest in the city states Hamburg and Berlin (73 and 116/100,000, respectively). Extrapolated to the SHI population, there were an estimated number of 176,181 LB/EM, 6,860 LNB and 4,509 LA cases in 2019. Mean excess all-cause costs per LB/EM patient in the first year following infection were €148 compared to matched controls and were highest in the first quarter after diagnosis. Excess costs in LNB patients were higher in all three years after diagnosis compared to matched controls (€3,249, €95 and €952 in years 1, 2, and 3, respectively). Excess costs in LA patients over controls were present in years 1 and 2 (€1,539 and €745, respectively). For all LB manifestations, there were more outpatient visits and longer hospital stays, compared to matched controls. Based on the number of extrapolated cases in 2019, additional SHI costs during the following year associated with LB/EM, LNB, and LA were around €26 million, €23 and €8 million, respectively.

**Conclusion**
We showed high LB incidence across all of Germany, including in states without mandatory surveillance systems. Healthcare costs for all LB manifestations add substantial economic burden to the overall German SHI system, with an estimated minimum of €57 million annual costs. However, LB incidence, and associated healthcare costs, in Germany may have been underestimated with our conservative case definition. Further research on societal costs particularly would provide a more complete picture of LB burden.
Experimental tick infections and comparative in vivo transmission studies confirm the vector competency of Dermacentor reticulatus ticks for tick-borne encephalitis virus.

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Tick biology, September 6, 2022, 2:30 PM - 3:50 PM

Introduction
Ixodes ricinus and I. persulcatus ticks are considered to be the main vector ticks of tick-borne encephalitis virus (TBEV; family Flaviviridae) in nature due to their specific ecological associations with the vertebrate hosts. However, several TBEV field studies in ticks suggest that Dermacentor reticulatus ticks might play a relevant role in the maintenance of TBEV in nature.

Aims
The goal of this study was to evaluate the vector competency of D. reticulatus for TBEV through experimental tick infections and comparative in vivo transmission studies involving D. reticulatus and I. ricinus ticks.

Methods
For experimental tick infections, unfed female ticks were infected with TBEV by micro-capillary inoculation into the coxal plate of the second pair of legs (transcoxal infection) using a digital microinjector system. Nymphs of D. reticulatus ticks were also infected by immersion in cell culture medium containing the virus.

For the transmission experiments, BALB/c mice were infested with a pair of either I. ricinus or D. reticulatus ticks (a virus-infected female and an uninfected male) together with 10 uninfected Haemaphysalis inermis nymphs held within retaining chamber attached to the dorsal surface of the trunk skin. Virus infection and/or transmission was evaluated by plaque assays and quantitative real-time RT-PCR.

Results
We observed that after a transcoxal micro-capillary inoculation, adult female D. reticulatus ticks efficiently replicated TBEV during the observed period of 21 days. The infected D. reticulatus ticks were able to transmit the virus to mice. The course of infection in mice was comparable to the infection after a tick bite by I. ricinus while the virus spread and clearance was slightly faster. Moreover, D. reticulatus ticks were capable of tick-to-tick non-viraemic transmission during co-feeding on the same animal. The co-feeding transmission efficiency was overall slightly lower (up to 54%) in comparison with I. ricinus (up to 94%) and peaked 1 day later, at day 3.

Conclusion
In conclusion, our study demonstrated that D. reticulatus is a biologically effective vector of TBEV. In line with the recent reports of its high TBEV prevalence in nature, our data indicate
that in some endemic foci, *D. reticulatus* might be an underrecognized TBEV vector which contributes to the expansion of the TBEV endemic areas.
Francisella tularensis exhibits distinct infection and replication kinetics in Amblyomma americanum and Dermacentor variabilis ticks

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Tick biology, September 6, 2022, 2:30 PM - 3:50 PM

Francisella tularensis is a highly-infectious bacterium and causative agent of the zoonotic disease tularemia. *F. tularensis* is classified as a Tier 1 Select Agent because of its low infectious dose (<10 CFU), ease of aerosolization, and high mortality rates (>60%). Although most U.S. tularemia cases are tick mediated, little is known about *F. tularensis* infections of ticks or transmission by infected ticks to humans. *Dermacentor variabilis* (American dog tick) has been reported to be primary tick vector for tularemia in the U.S., although *Amblyomma americanum* (lone star tick) also has been noted to transmit tularemia. Much remains to be learned about the tick vectors that pose the greatest risk for tularemia transmission and how *F. tularensis* infects, replicates, and is transmitted by ticks to hosts. We established a mouse-*F. tularensis*-tick infection model in our lab and infected both *D. variabilis* and *A. americanum* ticks with *F. tularensis* to directly compare tick infections over time and examine *F. tularensis* transmission by infected ticks to naïve mice. Despite equivalent *F. tularensis* numbers in infected mouse blood, replete *A. americanum* ticks contain one order of magnitude more *F. tularensis* than replete *D. variabilis* ticks, even after accounting for differences in blood volume per tick. Despite this initial difference, *F. tularensis* was found to replicate 20-fold in *D. variabilis* ticks during a 6-week incubation, compared to 1.1-fold replication in *A. americanum* ticks during the same incubation period. A second set of mouse-*F. tularensis*-tick infections demonstrated that *F. tularensis* infects and persists in both *D. variabilis* and *A. americanum* ticks for up to 14 weeks after feeding on infected mice, with higher *F. tularensis* numbers in *D. variabilis* ticks on week 14 (although not significant). On week 14, infected ticks were individually placed onto naïve mice and transmission efficiency was compared. Although not significant, 71% of *D. variabilis*-infested mice and 61% of *A. americanum*-infested mice became infected by *F. tularensis*. Taken together, these indicate that *D. variabilis* ticks may pose a greater health risk for transmitting tularemia. Ongoing studies are testing different infectious doses in both ticks, examining *F. tularensis* numbers when ticks are incubated at lower temperatures (simulating an overwintering event), and examining if tick microbiomes/endosymbionts are altered by *F. tularensis* infections.
mRNA vaccination targeting tick antigens induces resistance to *Ixodes scapularis*

*Dr. Erol Fikrig*

*Ixodes scapularis* ticks transmit many pathogens that cause human disease, including *Borrelia burgdorferi*. Acquired resistance to *I. scapularis* due to repeated tick exposure has the potential to prevent tick-borne infectious diseases, and salivary proteins have been postulated to contribute to this process. We examined the ability of lipid nanoparticle-containing nucleoside-modified mRNAs encoding 19 *I. scapularis* salivary proteins (19ISP) to enhance the recognition of a tick bite and diminish *I. scapularis* engorgement on a host and thereby prevent *B. burgdorferi* infection. Guinea pigs were immunized with a 19ISP mRNA vaccine and subsequently challenged with *I. scapularis*. Animals administered 19ISP developed erythema at the bite site shortly after ticks began to attach, and these ticks fed poorly, marked by early detachment and decreased engorgement weights. 19ISP immunization also impeded *B. burgdorferi* transmission in the guinea pigs. The effective induction of local redness early after *I. scapularis* attachment and the inability of the ticks to take a normal blood meal, suggests that 19ISP may be used either alone or in conjunction with traditional pathogen-based vaccines for the prevention of Lyme disease, and potentially other tick-borne infections.
Multidisciplinary Management of Suspected Lyme Borreliosis: Clinical Features of 569 Patients, and Factors Associated with Recovery at 12 Months, a Prospective Cohort Study

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Young & Wild: early career researchers’ presentations, September 5, 2022, 4:30 PM - 5:40 PM

Introduction
Because many patients with a suspicion of Lyme borreliosis (LB) experience difficult care paths, the Tick-Borne Diseases Reference Center (TBD-RC) was started in 2017. The aim of our study was to compare the clinical features of patients according to their final diagnoses, and to determine the factors associated with recovery in the context of multidisciplinary management for suspected LB.

Methods
We included all adult patients who were seen at the TBD-RC (2017-2020). Four groups were defined according to the European Study Group for LB (ESGBOR) and French guidelines: i) confirmed LB, ii) possible LB, iii) Post-Treatment Lyme Disease Syndrome (PTLDS) or sequelae, and iv) differential diagnosis. Their clinical evolution at 3, 6, and 9-12 months after care was compared and defined as so: complete recovery, partial improvement (persistent signs/symptoms allowing resumption of daily and professional activities), stagnation, or deterioration. Factors associated with recovery at 3 and at 9-12 months were identified using logistic regression models.

Results
Among the 569 patients who consulted, 72 (12.6%) had confirmed LB, 43 (7.6%) possible LB, 58 (10.2%) PTLDS/sequelae, and 396 (69.2%) a differential diagnosis. A favorable evolution was observed in 389/569 (68.4%) at 3 months and in 459/569 (80.7%) at 12 months, independent of the final diagnosis. Patients with partial improvement, stagnation, or deterioration presented associated diagnoses, explaining the absence of complete recovery. A longer delay between the first symptoms and the first consultation at the TBD-RC (p=0.001), the multiplicity of the diagnoses (p=0.004), and the inappropriate prescription of long-term antibiotic therapy (p=0.023) were negatively associated with recovery, reflecting serial misdiagnoses.

Conclusion
A multidisciplinary team dedicated to suspicion of LB may achieve a more precise diagnosis and better patient-centered medical support in the adapted clinical sector with a shorter delay, enabling clinical improvement and avoiding inappropriate antimicrobial prescription.
Oral Delivery of a Modern-Day Systemic Acaricide Formulation for Pathogen Vector Management on White-Tailed Deer in Connecticut, USA

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ECOLOGY, September 6, 2022, 10:50 AM - 12:50 PM

Introduction
Over twenty years ago, both ivermectin and doramectin were experimentally tested for management of parasitizing *Ixodes scapularis* and *Amblyomma americanum* on white-tailed deer (*Odocoileus virginianus*). While proven effective, there has been no further progress with this treatment strategy due to the required 48- and 45-day withdrawal periods (for use in cattle, respectively) for human consumption of treated animals. White-tailed deer are readily hunted in the fall, contemporaneously with peak activity of adult *I. scapularis*, rendering their systemic treatment impractical. However, moxidectin is a second-generation macrocyclic lactone with high lipophilicity, leading to superior bioavailability with a half-life > 20 days as opposed to 14 hours for ivermectin. Its labeled use in cattle (Cydectin® Pour-On for Beef & Dairy Cattle; Bayer Healthcare LLC, Shawnee Mission, KS, USA; FDA# 141-099) requires a 0-day withdrawal period; humans may consume meat or milk at any time post-treatment.

Aims
This was a proof-of-concept study to determine if we could orally deliver moxidectin to white-tailed deer and document levels previously reported lethal to ectoparasites.

Methods
Cydectin® was applied to whole corn at a dose of 10 mg moxidectin/0.45 kg. Treated whole corn was then distributed to two semi-contained white-tailed deer herds using automatic broadcast feeders at a rate of ~1 kg/animal/day during peak *A. americanum* nymphal and adult activity (May-June 2021) at one site (Norwalk) and during peak adult *I. scapularis* activity (November-December 2021) at another site (Bridgeport). Deer were captured via dart rifle and a blood sample obtained ~2, 4, and 6 weeks post-treatment and ~2 weeks after treatment had ceased. Sera were screened for moxidectin presence in parts per billion (ppb).

Results
In Norwalk, moxidectin was detected in 16 of 23 captures (70%) with mean = 27.3 (+ 6.0) ppb in those 16 moxidectin-positive captures. Additionally, deer positive for moxidectin were void of engorged *A. americanum*. In Bridgeport, moxidectin was detected in 16 of 21 captures (76%) with mean = 11.8 (+ 3.0) ppb in those 16 moxidectin-positive captures. We encountered several engorged *I. scapularis* females on moxidectin-positive animals during early capture events. We were successful in detecting moxidectin in 32 of 44 (73%) white-tailed deer captures in both locations combined.

Conclusion
We were successful in delivering moxidectin to wild white-tailed deer and achieved levels higher than reported effective in the literature (5-8 ppb). Leaner animals in spring had higher detectable free levels of moxidectin in their systems. We suspect spring moxidectin treatment of...
lean deer is taken up and metabolized rapidly as opposed to being stored in adipose tissue for longer-term release in fattier fall animals with slower metabolisms headed into winter. These metabolic differences should be taken into consideration when systemically treating white-tailed deer against ectoparasites with differing activity periods.
Tick-borne diseases (TBD) are on the rise, at least the number of newly detected assured or possible pathogens like Neo-Ehrlichia, Anaplasma or Borrelia (B.) miyamotoi. In face of the rapidly growing number of TBD’s diagnosis may be challenging.

Diagnosis of the different TBDs follows the same questions:
I. Epidemiology - was there any possibility of contact at all?
II. Are there symptoms that characterize the suspected disease?
III. Are there validated diagnostic measures to define the disease?

Reliable diagnosis based solely on epidemiological and clinical grounds is the exception.

Microbiological diagnostic measures are therefore important and may include direct detection by PCR, cultivation or microscopy and indirect by detection of specific antibodies. However, such tests are often not available or only available in specialized (research) laboratories.

Diagnosis of LB is based on epidemiology, objective clinical grounds and usually – except typical Erythema migrans - supported by microbiological diagnostics, especially serology. A challenge for microbiological tests is the heterogeneity of the *B. burgdorferi* sensu lato genospecies complex: at least seven genospecies found in Europe are assured or putatively pathogenic for humans.

Regarding antibody detection, a standard two-tier testing (STTT) is recommended in most European countries. For this a highly sensitive screening test (ELISA, CLIA,...) is performed that only in case of reactivity is confirmed by a confirmatory test, typically immunoblot (IB). In this setting the IB not only confirms the screening test but allows for assessment of the infection stage: in early disease typically IgM and/or IgG against OspC, VlsE and p41 are detectable, while in late stage disease the band pattern is much broader and includes antigens like p83/100, p58, p43 and further. As a rule, for late stage disease only *Borrelia*-specific IgG antibodies are of diagnostic value while negative or solely IgM-positivity argues against late manifestations.

Recently, modified STTT using two EIA’s showed superior sensitivity to STTT for diagnosis of early disease. Problematic issues regarding serology include: No acuity-marker, no therapy control, anamnestic titers and missing standardization.

Flanking microbiological diagnostics include PCR and cultivation from clinical material like CSF, biopsies or synovial fluid, all of which should only be performed after serology in still unclear cases. Further options include histology from skin biopsies, synovial fluid investigation and CSF investigations for intrathecal antibody production, signs of inflammation and local production of the B-cell attracting chemokine CXCL13.

Meanwhile there is a plethora of insufficiently validated tests available from specialized *Borrelia* or tick-borne disease laboratories. Not recommended diagnostics include Lymphocyte transformation test, Enzyme-linked Immunospot Assay, PCR from blood or urine, dark-field microscopy directly from patient material, detection of so-called L-forms or spheroplasts, CD57-positive/CD3-negative lymphocyte subpopulation, HLA-determination, prophage-diagnostics, or diagnostics without clinical indication.
Successes and continued challenges
Dr. Linden Hu

September 4, 2022, 05:40 PM - 06:10 PM

Tick-borne Diseases 2022—Successes and continued challenges

Tick-borne diseases continue to expand in both numbers and geographic distribution with at least 7 new tick-vectored human pathogens identified in the U.S. in the last decade. During this presentation, we will discuss progress and ongoing challenges to the diagnosis, prevention and treatment of tick-borne diseases. We will discuss the significant advances in the direct detection of many tick-borne pathogens and contrast that with the diagnosis of other tick-borne diseases that continue to rely on older indirect technology. During the presentation, we will review the many new, innovative approaches targeting either humans or the natural reservoirs to prevent tick-borne disease. Notably, the majority remain at the early stages of development and will likely be years before they make an impact on the incidence of human tick-borne disease. Finally, hurdles surrounding the lack of progress for development of new treatments or treatment regimens for tick-borne diseases will be reviewed.
Borrelia burgdorferi is a stealth bacterium and, in general, lack classical virulence factors associated with invasive pathogens. As such, the mechanisms that underlie Lyme disease are largely unknown. One example of this quagmire is Lyme arthritis—the proliferative synovitis of one or more large joints—which can occur after extensive antibiotic therapy and in the absence of a detectable infection. We have discovered that B. burgdorferi sheds ~45% of its peptidoglycan (PG)—the essential component of the bacterial cell-wall—from inside the cell, into its environment. B. burgdorferi PG can be detected in the synovial fluid of Lyme arthritis patients’ months after oral and/or intravenous antibiotics. When injected into a mouse, B. burgdorferi PG alone, is capable of causing arthritis. Virtually all bacteria have PG but, as it turns out, B. burgdorferi PG is chemically unique and unlike any previously studied. For instance, B. burgdorferi PG peptides contain Ornithine-Glycine rather than Lysine or Diaminopimelate. PG containing Ornithine has been reported in other spirochetes that do not induce arthritis and cannot, alone, explain our earlier findings. Using high-resolution LCMS methods, coupled with metabolic labeling studies and NMR, we discovered that B. burgdorferi PG contains the unprecedented trisaccharide GlcNAc-GlcNAc-MurNAc, which is released during growth. This modification to PG glycans can be partially explained by acquisition and incorporation of the tick-vector sugar chitobiose (GlcNAc-GlcNAc). Atomic force microscopy studies reveal that GlcNAc-GlcNAc-MurNAc glycan organization is a novel means to 1) increase distance between stem peptides; 2) produce more flexible PG; and 3) collectively optimize motility to withstand the torque of endoflagella. From a pathogenesis standpoint, real-time PG tracking experiments in live animals, in conjunction with single-cell RNA-seq, suggest that the unique features of B. burgdorferi muropeptides contributes to half-life in discrete tissues and an unusual immunological response that is dependent on the atypical features of the B. burgdorferi cell wall.
The rise of urban tick-borne diseases: the roles of greenspace connectivity and wildlife community assembly

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Ecology, September 6, 2022, 10:50 AM - 12:50 PM

Introduction
Biodiversity loss has been proposed as a driving force for the emergence of zoonotic diseases, mediated by hunting pressure and fragmentation causing population declines. In North America, the emergence of tick-borne diseases has been considered a prime case study for a negative biodiversity-disease relationship (the ‘dilution effect’). The proposed mechanism is an increase in enzootic hazard (the density of infected ticks) in smaller and more isolated forest fragments due to reduced biodiversity and increased dominance of reservoir competent small mammal hosts. In contrast to this expectation, we previously found that extreme patch isolation in New York City resulted in failed tick establishment due to limited accessibility for white-tailed deer, the reproductive and keystone host for three tick vector species (Ixodes scapularis, Amblyomma americanum and Haemaphysalis longicornis). Furthermore, fragmented landscapes have been sometimes shown to have higher host species diversity due to anthropogenic resource subsidies. We hypothesized that the likelihood of detecting three tick vectors species (I. scapularis, A. americanum and H. longicornis) in urban parks and adjacent residential yards in NYC was associated with (1) landscape connectivity, (2) patterns of host community assembly and (3) yard-level habitat suitability for ticks and its wildlife attractive (e.g. food, shelter) or repellent (e.g. fencing) features.

Methods
During Summer 2021, we sampled five neighborhoods on Staten Island, a borough of New York City. In paired yards and adjacent natural areas, we sampled ticks using standard dragging techniques, hosts using camera traps and hair tubes and recorded multiple yard-level features. We characterized landscape patterns using Fragstats and other tools. We used generalized linear models to determine the host community composition, and residential yard- and landscape-level features associated with the presence of three tick species.

Results
(1) Landscape connectivity: The amount and configuration of canopy cover surrounding residential yards strongly predicted the presence of I. scapularis and A. americanum, but not that of H.s longicornis. (2) Host community assembly: All host species were present in natural areas; the subset of hosts most commonly occurring in yards included squirrels, raccoons, domestic cats, and white-tailed deer, all considered pathogen ‘dilution’ hosts; the number of days deer were detected in the yard predicted the presence of I. scapularis and A. americanum. (3) Yard-level features: Fencing had
limited protective effect; the presence of log and brush piles increased the odds of finding all species ticks in yards.

**Conclusion**
Landscape connectivity and the presence of white-tailed deer enhanced the establishment of ticks and tick-borne pathogens in NYC. All reservoir hosts were present in all studied urban parks; the subsets of hosts in yards were dominated by ‘dilution’ rather than highly competent hosts.
Tick-borne encephalitis in the Åland Islands 2006-2020: incidence and clinical characteristics since implementation of mass vaccination.

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Clinical aspects, September 5, 2022, 2:30 PM - 4:00 PM

Introduction
Tick-borne encephalitis (TBE) is one of the most serious infectious diseases transmitted by ticks and is emerging in Eurasia and in the Nordic countries, of which the Åland Islands are highly endemic. Wahlberg et al. (1) characterised TBE cases on the islands 1959-2005. This study is a retrospective follow-up of TBE cases on Åland 2006-2020, since start of mass vaccination in 2006.

Methods
Children <18 years and adults with TBE diagnosed in the Åland Islands 2006-2020 were included. Medical records were reviewed and the diagnosis of TBE was re-evaluated according to diagnostic criteria. Vaccination status was clarified, and the disease severity and outcome were estimated.

Results
103 patients (68% males, median age 55 years, 8.7% were children) were diagnosed with TBE. The incidence was 23/100 000/year, with a peak in September (28%). In 53% of the subjects, the first health care contact was in the primary care setting. 15 (16%) were fully vaccinated and 53 (55%) were unvaccinated. 39 (38%) were diagnosed with meningitis and 64 (62%) with meningoencephalitis with a biphasic disease course in 47 (46%). Nine patients (8.7%) needed intensive care. Disease severity correlated with increasing age. No fatal cases occurred.

Conclusion
Compared to the years before vaccination, the incidence of TBE has not significantly decreased or increased in the last 15 years. Vaccination breakthroughs occurred, although >50% of the cases were unvaccinated. TBE on Åland still entails high costs for healthcare and causes personal suffering due to sequelae.

Treatment of Erythema Migrans With Doxycycline for 7 Days Versus 14 Days: a Noninferiority Randomized Open-Label Study

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Clinical aspects, September 5, 2022, 2:30 PM - 4:00 PM

Introduction

With awareness of increasing bacterial resistance globally, it is important to re-evaluate the duration of therapies needed for particular infections. Doxycycline for 10 days is the shortest treatment duration recommended for adult patients with erythema migrans, but shorter regimens have not been assessed.

Methods

In an open-label randomized clinical trial, performed at the University Medical Centre Ljubljana, Slovenia, the efficacies of 7-day versus 14-day of oral doxycycline therapy were compared on a noninferiority premise in adult patients with solitary erythema migrans. The efficacy of treatment was assessed based on clinical and microbiologic parameters, assessed at 14 days and at 2, 6, and 12 months after enrolment.

Results

Out of 300 randomized patients, 147 patients (50.5%) completed treatment with doxycycline for 7 days, and 144 patients (49.5%) received doxycycline for 14 days. Patients in the two treatment groups did not differ regarding basic demographic, clinical, and microbiologic characteristics at enrolment. The proportion of patients with incomplete response decreased during follow-up, and was comparable between 7-day and 14-day treatment groups (14 days: 25/144 [17.4%] vs 29/141 [20.6%]; P=0.295; 2 months: 27/136 [19.9%] vs 23/132 [17.4%]; P=0.638; 6 months: 15/118 [12.7%] vs 14/124 [11.3%]; P=0.557). At the 12-month visit, 8/101 [7.9%] patients in the 7-day vs 9/102 [8.8%] patients in the 14-day group showed incomplete response (difference -0.9 percentage points; 1-sided 95% CI, -1 to 0.06 percentage points; P=0.5). None of the patients developed new objective manifestations of Lyme borreliosis during follow-up and none had positive skin re-biopsy culture result for borreliae.

Conclusion

The 7-day regimen of oral doxycycline was noninferior to the 14-day regimen for treating adult European patients with solitary erythema migrans. At 12 months post-enrolment, only a minority of patients had incomplete response, manifested as post-Lyme borreliosis symptoms.
Understanding mechanism underlying Lyme borreliosis and its post-treatment sequelae

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Pathogenesis, September 6, 2022, 09:00 AM - 09:30 AM

Background

Patients with Lyme borreliosis may present with a range of clinical manifestations which vary in severity and duration, including complications which persist after antibiotic therapy for the infection. The reasons for this heterogeneity are not well understood, but host immune responses are thought to play an important role. Herein we focus on our recent longitudinal study of immune responses in patients with Lyme neuroborreliosis (LNB), approximately 20% of whom may experience symptoms that persist despite appropriate antibiotic therapy, including pain, fatigue, and neurocognitive deficits.

Methods

Seventy nine patients with LNB, 28 with persistent symptoms and 51 without, were followed systematically from the initial visit prior to antibiotic therapy for 1 year thereafter to evaluate their clinical course and outcome. Patients with lingering symptoms and those without were evaluated by clinical, laboratory, and demographic characteristics. In addition, the levels of 20 inflammatory mediators associated with innate and adaptive immune responses were determined in matched serum and cerebrospinal fluid (CSF) to test the possibility that lingering symptoms after LNB are due to maladaptive immune responses triggered during the infection.

Results

At study entry, most inflammatory mediators were highly concentrated in CSF, the site of the disease. These responses resolved with antibiotic therapy, and the associations between CSF cytokines and signs and symptoms of LNB were no longer observed. In contrast, symptoms that persisted after antibiotics were associated with elevated IFNα levels in serum, which were already observed at study entry (median=18 vs 2 pg/ml, p=.01) and remained elevated at each subsequent timepoint. When stratified by severity, patients with the most severe post-Lyme symptoms which required regular use of analgesics had the highest levels of IFNα, whereas those whose symptoms resolved had the lowest IFNα levels (p=0.006). Finally, post-treatment symptoms occurred more frequently in women, and in those with a highly symptomatic preceding infection (odds ratio=4.8), but were not associated with borrelia culture positivity or with other clinical, laboratory, or immunologic abnormalities in CSF. These findings imply that although the infection serves as the initial trigger, post-LNB complications likely result from sustained maladaptive immune responses in blood.

Conclusion

Debilitating sequelae after LNB are associated with unremitting systemic levels of IFNα, consistent with the pathogenic role of this cytokine in Type1 interferonopathies in other infectious and autoimmune conditions. The emergence of data supporting the role of immune response in post-Lyme sequelae may provide new considerations for treatment approaches which
prioritize targeting the immune response after appropriate antibiotic regimens, a treatment algorithm currently used effectively in treatment of post-antibiotic Lyme arthritis.
Unique Clinical, Immune, and Genetic Signature in Patients with Borrelial Meningoradiculoneuritis

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Clinical aspects, September 5, 2022, 2:30 PM - 4:00 PM

Background
Lyme neuroborreliosis (LNB) is the most common extra-cutaneous manifestation of Lyme borreliosis in Europe. LNB most often presents as painful meningoradiculoneuritis, also known as Bannwarth’s syndrome or lymphocytic meningitis with or without cranial neuritis. Although these conditions represent separate clinical entities, their pathophysiology is not well understood and the possibility that they involve distinct immune or genetic phenomena has not been tested.

Methods
Herein we assessed host immune responses and the prevalence of single nucleotide polymorphism in the TLR1 (toll-like receptor 1) gene, the key sensor for Borrelia, in 61 patients with confirmed LNB, 19 with Bannwarth’s syndrome and 42 with cranial neuritis or lymphocytic meningitis, and 59 patients with suspected LNB. The levels of 17 cytokines and chemokines associated with innate and adaptive immune responses were determined in matched serum and cerebrospinal fluid (CSF) samples using Luminex bead-based assays. The frequency of TLR1-180GG polymorphism was determined by PCR followed by restriction fragment length polymorphism.

Results
In all LNB patients, regardless of manifestation, most immune mediators were concentrated in CSF, the site of the disease, implicating the immune response in disease pathogenesis. In contrast, the levels of these mediators in serum were unremarkable. Lowest levels of inflammatory mediators were observed in patients with suspected LNB who lacked CSF pleocytosis. When stratified according to specific clinical presentation, patients with Bannwarth’s syndrome had markedly elevated levels of B cell chemoattractants CXCL13 (median 1000pg/mL vs 53pg/mL; \( P=.02 \)) and CXCL12 (median 2568pg/mL vs 1170pg/mL; \( P=.05 \)), and T cell-associated mediators CXCL9, CXCL10, and IL-17, in CSF compared to those with LNB without radicular pain. Moreover, they had a higher frequency of TLR1-1805GG polymorphism (68% vs 44%; OR=3.1, \( P=.03 \)), and a greater number of constitutional symptoms, consistent with the previously defined role of this polymorphism in highly symptomatic early infection in patients with erythema migrans.

Conclusions
Bannwarth’s syndrome is associated with a greater frequency of TLR1-1805GG polymorphism, heightened B and T cell inflammatory responses in CSF, and a more symptomatic disease course compared to other manifestations of LNB, or suspected LNB. These findings demonstrate that Bannwarth’s syndrome is a distinct clinical entity with unique immune and genetic pathophysiology and provide a new paradigm for the study of borrelial meningoradiculitis and LNB in general.
Utility of Commercial Insurance Claims to define the Incidence of Lyme disease During Pregnancy

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Introduction
Pregnant women are particularly sensitive to certain infections. The frequency and consequences of Lyme disease among pregnant women is poorly described. While there is some evidence that transplacental transmission can occur, epidemiologic studies of Lyme disease and pregnancy outcomes have been limited and the results inconsistent. Commercial health insurance claims are a large, high-quality data source that could provide new insights into both the frequency and impacts of Lyme disease during pregnancy.

Methods
We used previously published methodologies to identify pregnancies and Lyme disease diagnoses within the IBM® MarketScan® Commercial Claims and Encounters Databases, covering >25 million insured United States residents aged <65 years. We extracted specific inpatient and outpatient diagnosis, procedure, and diagnosis-related group (DRG) codes that occurred between January 2011 and October 2015 from records of females aged 12-55 years to identify pregnancies and assign their outcomes (live birth, stillbirth, elective abortion, spontaneous abortion, abortion of unknown type, or ectopic pregnancy). We then identified those pregnancies where a maternal Lyme disease diagnosis occurred anytime between the last menstrual period and the end of pregnancy. These data were used to estimate the incidence of Lyme disease among this pregnant, insured population and the frequency of outcomes for the pregnancies coincident with a Lyme disease diagnosis.

Results
A total of 2,575,614 pregnancies were identified within the covered population, of which 252 (~16 per 100,000 pregnancies per year) were coincidentally diagnosed with Lyme disease. Pregnancy outcome rates were not significantly different (p=0.07) among those with and without a coincident Lyme disease diagnosis: 85% vs 78% live birth, 15% vs 22% abortion (spontaneous, elective, or unspecified), and 0.0% vs 0.4% stillbirths, respectively.

Conclusions
Insurance claims from a large population are useful to identify pregnancies with coincident Lyme disease and understand potential impacts of the illness on those pregnancies. In these preliminary analyses, the observed incidence of Lyme disease during pregnancy appeared to be somewhat lower than what has been published (~30-50 per 100,000 per year) for the similarly aged female MarketScan® population during this time period, although small numbers of Lyme disease diagnoses in this study population make comparisons challenging. We also did not observe higher rates of pregnancy loss (spontaneous, elective, or unspecified) or stillbirth among women diagnosed with Lyme disease compared to the general pregnant population. Additional analyses are underway to confirm these findings and to estimate the risk for adverse birth outcomes, including malformations.
Chronic B. burgdorferi s.l. infection: lessons from animal models.

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Lyme in the Lymelight, September 6, 2022, 05:10 PM - 05:30 PM

Chronic B. burgdorferi s.l. infection: lessons from animal models. Lack of efficacy of antibiotic treatment in Lyme disease may be attributed to several causes, including (1) spirochetes that persist in the tissues, tolerant to the antibiotic used; (2) inflammatory responses to residual antigens from dead organisms; or (3) autoimmune responses, possibly elicited by antigenic mimicry. The mouse model has been utilized in several studies of antibiotic efficacy for treatment of early and established B. burgdorferi infections. “Persisters,” a small subpopulation of bacteria that are dormant and non-dividing, have been documented since 1944 during early studies on the action of penicillin on Staphylococcus spp., and have subsequently been found among a wide variety of other bacteria. Persisters are tolerant (in contrast to resistant) to antibiotics because they have little or no cell wall synthesis, translation or topoisomerase activity. Although not distinctly proven in vivo with B. burgdorferi, studies show that the spirochetes do form antibiotic-tolerant persisters in vitro. This may explain the reduced effectiveness of antibiotic therapy during late Lyme disease, and results from several studies in animal models (dogs, non-human primates and mice) in which spirochetes cannot be cultured after antibiotic treatment, but can be detected in tissues by PCR and RT-PCR, suggest persister forms. Live and non-dividing populations of persisting spirochetes can be detected in antibiotic-treated mice and primates by xenodiagnosis. Intact Borrelia have also been detected by immunofluorescent staining in a variety of tissues from mice and primates subsequent to antibiotic treatment. These results demonstrate that B. burgdorferi can withstand antibiotic treatment and persist in tissues post-dissemination, raising important questions about the pathogenicity of antibiotic-tolerant persisters and whether or not they can contribute to symptoms of PTLDS.
Molecular mechanisms of tick-borne encephalitis virus neutralization by monoclonal antibodies

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New and Hot, September 5, 2022, 09:20 PM - 09:40 PM

Invited lecture:
Tick-borne encephalitis (TBE) is a potentially lethal neuroinfection in humans, caused by TBE virus (TBEV), a member of genus Flavivirus, family Flaviviridae. The disease is prevalent in forested areas of Europe and northeastern Asia. Specific anti-TBEV immunoglobulin is currently used with well-documented effectivity for post-exposure prophylaxis and TBE treatment in Russia and Kazakhstan, but the use of specific TBEV immunoglobulins has been discontinued in Europe due to concerns regarding antibody-dependent enhancement (ADE) of infection in naïve individuals. However, the mechanism of TBEV antibody-mediated neutralization and/or ADE remains unknown. Here, we determined cryo-EM structures of the native TBEV virion (strain Hypr, European TBEV subtype) and its complex with Fab fragments of neutralizing antibody 19/1786 at near-atomic resolution. The Fab fragments bind to 120 out of the 180 envelope glycoproteins of the TBEV virion. Unlike most of the previously studied flavivirus-neutralizing antibodies, the Fab fragments do not lock the E-proteins in the native-like arrangement, but prevented the virus proteins from inducing membrane fusion in the endosome and releasing the viral nucleocapsid into the cytoplasm. Because the IgG 19/1786 antibody is not cross-reactive against other flaviviruses and efficiently neutralizes TBEV, it has potential for therapeutic use in patients with TBE. Analysis of human antibody response to TBEV infection or vaccination revealed that expanded clones of memory B cells expressed closely related anti-envelope domain III (EDIII) antibodies in both cohorts, but the most potent neutralizing antibodies were found only in individuals who recovered from natural infection. These antibodies also neutralized other tick-borne flaviviruses. Structural analysis revealed a conserved epitope near the lateral ridge of EDIII adjoining the EDI-EDIII hinge region. Prophylactic or early therapeutic antibody administration was effective at low doses in mice lethally infected with TBEV. Antibody-resistant TBEV mutants were generated and characterized. The mutants had amino acid substitutions in EDIII and EDII, showed a small plaque size in mammalian cell culture and reduced levels of neuroinvasiveness in rodent models compared to the wild-type TBEV. The results demonstrate the importance of critical sites within the EDIII as determinants of virus virulence.
Poster abstracts

P001 - Tick infestation in Phyllotis darwini (Rodentia) in an anthropogenic landscape of the semi-arid Chile

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Introduction
A series biotic and abiotic modifications occur in an anthropogenic landscape which influence the ecology and physiology of wild hosts and their parasites. How these changes affect the parasitism is highly context-dependent, based on the host-parasite system and the type of perturbation. We aimed to investigate the differences in infestation with ticks of the native rodent Phyllotis darwini in two areas with different anthropogenic impacts and to evaluate environmental and host factors associated with tick infestation.

Methods
We studied wild and rural sites of the semiarid Coquimbo region of Chile in the austral spring and summer seasons in 2018-2019, 2019-2020 and 2021-2022. We live-trapped and chemical immobilized wild rodents in four grids in each wild (Fray Jorge National Park) and rural sites (El Tangue ranch). During five minutes rodents were combed and ticks removed and deposited in ethanol for further molecular analyses. Host (age, sex) and environmental (Season, Site, relative humidity and temperature) were obtained and then we used Generalized Linear Mixed Models and Generalized Linear Models to test factor effects on the tick infestation of ticks parasitizing adult rodents. All statistical analyses were carried out in R.

Results
Overall, out of 446 rodents trapped, 15.9% showed tick infestation. All ticks were larvae or nymphs of Ixodes sp. In the wild and rural sites 257 and 189 rodents were trapped, with an infection rate of 20.2% and 10.1%, respectively. On the other hand, rodents trapped in spring and summer were 288 and 158, respectively showing an overall increase in the infection rate from 9.0% in spring to 28.5% in summer. When comparing the results between years an increase in tick infestation rate was found from the period 2018-2019 (17.9%, 30/168) compared with the period 2019-2020 where the highest rate was found (32.8%, 19/58) and finally a reduction in the infection rate for the period 2021-2022 (10.0%, 22/220) was detected. Thus, rodents showed statistically a higher infestation probability in summer months, higher in the wild than the more anthropized rural site and a variation across year, increasing in the period 2019-2020.

Conclusions
Environmental factors such as seasonality, site and year of sampling are associated to tick infestation rate in wild rodents in Coquimbo region in Chile. Seasonality is likely associated to temperature restriction of ticks during their lifecycle. On the other hand, the lower infestation rate in anthropized sites could be associated to a reduced number of hosts for tick development. Finally, yearly fluctuations are likely to be associated to a combination of biotics (eg. Population density) and abiotic (drought in 2019) factors. This study was funded by projects Fondecyt Nº 1180119 and 1211190 and by ANID/CONICYT Doctoral Grant Nº 21171018 to Esperanza Beltrami.
P002 - First report of Theileria annulata in Nigeria: Findings from cattle ticks in Zamfara and Sokoto States.

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Introduction
Ticks and tick-borne pathogens (TBPs) represent a significant economic burden to cattle farming in sub-Saharan Africa including Nigeria. However, in the northern part of this country, where the largest livestock population resides, little is known about the contemporary diversity of ticks and TBPs, with the majority of the studies detecting the presence of apicomplexan parasites relying on poorly sensitive cytological and serological assays. This area is particularly vulnerable to climate change, undergoing marked transformation of habitat and associated flora and fauna that is also likely to include ticks.

Aims
This study aimed to document the diversity of cattle-associated ticks and apicomplexan TBPs in a region of north-western Nigeria heavily reliant on cattle keeping and significantly affected by climate change.

Methods
In 2017, ticks were collected from cattle in Zamfara and Sokoto States and identified morphologically to the species level. Additionally, a subset of ticks was screened molecularly for the detection of apicomplexan DNA.

Results
A total of 494 adult ticks were collected from 80 cattle in Zamfara and 65 cattle in Sokoto State. Nine tick species were encountered, among which the presence of one, Hyalomma turanicum, had not previously been recorded in Nigeria. Hyalomma rufipes was the most prevalent tick infesting cattle in Zamfara State (76%), while Hyalomma dromedarii was the most prevalent in Sokoto State (44%), confirming the widespread transfer of this species from camels onto livestock and its adaptation to cattle in the region. Of 159 ticks screened, 2 out of 54 (3.7%) from Zamfara State and 29 out of 105 (27.6%) from Sokoto State harboured DNA of Theileria annulata, the agent of tropical theileriosis.

Conclusion
This survey confirms the presence of a broad diversity of tick species in cattle from north-western Nigeria, providing the first locality records for Zamfara State. The occurrence of H. turanicum indicates a distribution of this tick beyond northern Africa. This study provides the first report for T. annulata in Nigerian ticks. Given the enormous burden of tropical theileriosis on livestock farming in north Africa and across Asia, further investigations are needed to better understand its epidemiology, vector transmission and potential clinical significance in cattle from northern Nigeria and neighbouring Sahelian countries.
P003 - Characterising the Borrelia of Ornithodorous savignyi ticks

Adefolake Bankole, Prof Kumsa Eseta Berssisa, Dr Ndudim Ogo, Dr Nusirat Elelu, Prof Winston Morgan, Prof Sally Cutler

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Adefolake Abstract 13-5-22 (could not be inserted)
The murine Lyme arthritis immunopeptidome: linking infection, arthritis, and autoimmunity

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Introduction
Immune dysregulation in response to infectious agents may trigger autoimmunity but establishing a causal relationship between a pathogen and autoimmune disease has been immensely challenging. Lyme arthritis (LA), which is caused by infection with Borrelia burgdorferi (Bb), is frequently accompanied by autoimmune B and T cell responses in humans, resulting in chronic inflammation. In this study, we used immunopeptidomics and histology to gain further insight into mechanisms of immune evasion and infection-induced autoimmunity by using two mouse models: C57BL/6 (B6) mice, which develop mild inflammatory, self-resolving LA, and B6 Il10⁻/⁻ (IL-10 KO) mice, which develop severe, autoimmune-like LA.

Methods
B6 and IL-10 KO mice were inoculated with 2x10³ Bb. Joint-draining inguinal and popliteal lymph nodes (LN) and tibiotarsal joints (TJ) were harvested at 4 and 16 weeks post-inoculation. MHC class II molecules were isolated from LN by immunoaffinity capture and MHC-bound peptides were identified by LC-tandem MS. TJs were stained with H&E, Masson’s trichrome, or anti-CD31.

Results
Nearly 10,000 MHC-II peptides were identified by LC-MS/MS. Peptides derived from extracellular matrix (ECM) components (collagens, laminins, and fibronectin) that are targets in other autoimmune diseases, were abundant. Notably, the number of Apolipoprotein B-100 (ApoB-100) peptides, a human Lyme autoantigen, was greatly increased in LNs of infected mice, particularly in IL-10 KO mice at 4 weeks (6-fold increase) and 16 weeks (15-fold increase) post-infection, compared with uninfected mice. H&E and Masson’s trichrome histology of TJs showed increased inflammatory infiltrate and fibrosis in infected IL-10 KO mice compared to infected B6 mice. Anti-CD31 staining of endothelial cells showed marked neovascularization. Infected IL-10 KO mice synovial tissue showed obliterative microvascular lesions, as observed with human LA. Surprisingly, only six peptides derived from Bb proteins were identified in this study; all Bb peptides were from proteins localized to the inner membrane or cytosol, and none were from outer surface lipoproteins.

Conclusion
This proteomics study provides a profile of immune-relevant proteins associated with LA development. Expansion of peptides derived from ECM proteins and proteins associated with vascular damage, such as ApoB-100, in draining LNs corresponded with histopathological evidence of ECM remodeling and vascular involvement within inflamed joint tissue. Notably, many peptides were derived from known autoantigens in other diseases, including rheumatoid arthritis, LA, and atherosclerosis. Together, these data provide a critical link between B. burgdorferi infection, LA, and development of infection-induced autoimmunity.
Prevalence of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks and the impact of two climatic factors

Mrs Teodora Gladnishka, Prevalence of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks and the impact of two climatic factors Iva Christova, Prevalence of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks and the impact of two climatic factors Iva Trifonova, Prevalence of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks and the impact of two climatic factors Vladislava Ivanova, Prevalence of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks and the impact of two climatic factors Elitsa Panayotova, Prevalence of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks and the impact of two climatic factors Evgenia Taseva

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Introduction
Climate conditions strongly influence tick population density and activity as well as occurrence of tick-borne diseases. Ticks are constantly expanding their range of distribution, which is associated with global warming. Lyme borreliosis is the most common tick-borne infection in North America and Europe. The main vector is Ixodes ricinus tick. It is known that the ticks are susceptible to climatic determinants, specifically temperature and precipitations.

The aim is to investigate infection with Borrelia burgdorferi sensu lato in ticks removed from patients and abundance of ticks in relation to temperature and precipitations in 2016-2021 in Sofia, Bulgaria.

Methods
A total of 9,564 ticks were collected from patients in 2016-2021. They were identified to the species and phase of development. Nested PCR was performed targeting two regions of the spacer region between 5S and 23S of B. burgdorferi s.l. rRNA. The weather data were received from Internet free meteorological sites. Diagrams were made for distributions of the ticks by months and for the infection with B. burgdorferi s.l. throughout the six years. Data on the number of ticks were analyzed against average temperature and precipitations during the study period.

Results
It was found that 96% of ticks belonged to the species I. ricinus. The highest number of ticks in May was observed in 2018, and the peaks in the other five years of the study were found in June. The highest tick infection with B. burgdorferi s.l. was detected in 2019-25.32%, and the lowest in 2018-10.15%. Nymphs (15.84%) were less infected than females (20.35%) only in 2016. An increase in tick number was observed at average temperatures around 20°C, with rainfall on the days before the peak. Spring with high average temperatures and a lot of rain in April-June led to the highest number of ticks in June 2021 during the 6-year period.

Conclusions
Temperature and precipitations were found to be factors influencing I. ricinus abundance and activity. Data of prevalence of B. burgdorferi s.l. in I. ricinus ticks and weather factors may be useful in connection with the deacarisation of lawns in the city parks, where the risk of human infection with B. burgdorferi s.l. spirochetes is the highest. This applies in particular to highly anthropophilic nymphs, number and infection of which increased during the study period. The study is valuable with the monitoring of climatic factors-temperature and precipitations, which indicate that number and level of infestation of I. ricinus ticks might be taken into account for the risk assessment of Lyme borreliosis.
P006 - Questing I. scapularis ticks maintained for one year under optimal environmental conditions transmit B. burgdorferi to uninfected mice

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Introduction
In recent decades, Lyme disease has been expanding to previous non-endemic areas.

Aims (optional)
We hypothesized that infected I. scapularis nymphs that retain host-seeking behavior under optimal environmental conditions are fit to fulfill their transmission role in the enzootic cycle of B. burgdorferi.

Methods
To test this hypothesis, we produced nymphal ticks in the laboratory under controlled temperature (22-25°C), humidity (80-90%) and natural daylight cycle conditions to allow them to retain host-seeking/questing behavior for ~1 year. We then analyzed differences in B. burgdorferi infection prevalence in questing and diapause nymphs at 6 weeks post-molting (prime questing) as well as differences in infection prevalence of questing nymphs maintained under prolonged environmental induced questing over 12 months (prolonged questing). Lastly, we analyzed the fitness of nymphal ticks subjected to prolonged questing in transmission of B. burgdorferi to naïve mice over the course of the year.

Results
Our study shows that infected unfed I. scapularis nymphal ticks maintained under optimal environmental conditions in the laboratory not only survived for a year in a developmental state of prolonged questing (host-seeking), as they retained an infection prevalence sufficient to effectively fulfill transmission of B. burgdorferi to uninfected mice after tick challenge.

Conclusion
Our study is important for understanding and possibly modeling Lyme disease expansion into former non-endemic regions due to global warming.
P007 - Identification of tick Ixodes ricinus midgut genes differentially expressed during the transmission of Borrelia afzelii spirochetes using a transcriptomic approach

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Lyme borreliosis is an emerging tick-borne disease caused by spirochetes Borrelia burgdorferi sensu lato. In Europe, Lyme borreliosis is predominantly caused by Borrelia afzelii and transmitted by Ixodes ricinus. Although Borrelia behavior throughout tick development is quite well documented, specific molecular interactions between Borrelia and the tick have not been satisfactorily examined. Here, we present the first transcriptomic study focused on the expression of tick midgut genes regulated by Borrelia. By using massive analysis of cDNA ends (MACE), we searched for tick transcripts expressed differentially in the midgut of unfed, 24h-fed, and fully fed I. ricinus nymphs infected with B. afzelii. In total, we identified 553 upregulated and 530 downregulated tick genes and demonstrated that B. afzelii interacts intensively with the tick. Technical and biological validations confirmed the accuracy of the transcriptome. The expression of five validated tick genes was silenced by RNA interference. Silencing of the “uncharacterized protein” delayed the infection progress and decreased infection prevalence in the target mice tissues. Silencing of other genes did not significantly affect tick feeding nor the transmission of B. afzelii, suggesting a possible role of these genes rather in Borrelia acquisition or persistence in ticks. Identification of genes and proteins exploited by Borrelia during transmission and establishment in a tick could help the development of novel preventive strategies for Lyme borreliosis.

Supported by: Centre for Research of Pathogenicity and Virulence of Parasites (no. CZ.02.1.01/0.0/0.0/16_019/0000759), funded by the European Regional Development Fund (ERDF) and Ministry of Education, Youth, and Sport, Czech Republic (MEYS).
P008 - Decorin binding proteins from European Borrelia – do structural differences influence ligand binding?

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Adhesion of spirochetes from *Borrelia burgdorferi* sensu lato complex is the crucial step in early phase of Lyme disease infection. Decorin binding proteins (Dbp) are glycosaminoglycan (GAG) binding adhesins exposed on the surface of borrelia spirochetes. Dbps are expressed in two homologous forms A and B, both were characterized as main factors of borrelia virulence. Based on the previous described differences in binding mechanisms of Dbp-GAG interaction, we focused on the relations between structural differences of Dbps across borrelia species and GAG binding. We aim to describe the structural differences in detail among Dbps from European borrelia species and their particular interactions with different GAGs using solution nuclear magnetic resonance (NMR) spectroscopy. Almost complete backbone and sidechain assignments of DbpA from *B. Afzelii* and *B. Bavariensis* have been achieved. Predictions of secondary structure propensity for both variants (TALOS N) were compared with available NMR structures of North American borrelia species. Backbone dynamics was described by T\(_1\) and T\(_2\) spin relaxations and \(^1\)H \(^{15}\)N heteronuclear NOE (Nuclear Overhauser effect) experiments. We performed initial protein-GAG interaction studies of both variants of DbpA with different GAGs by NMR titrations. In addition, we have followed changes to the local backbone dynamics via \(^1\)H \(^{15}\)N heteronuclear NOE, hydrogen-deuterium exchange mass spectrometry (HDX-MS) and surface plasmon resonance (SPR) trial measurement. NMR-based prediction of secondary structure propensity and protein backbone dynamics combined with initial protein-ligand interaction experiments indicates interspecific differences in GAG binding. The prediction calculations provided insight into structural characteristics of DbpA. This obtained data will set the starting point for future extensive research of specific differences in structure and dynamics of Dbps and how it influences the interaction mechanism with GAG ligands.
Introduction

Bacteria of the *Borrelia burgdorferi* sensu lato species complex are the causative agents of Lyme borreliosis and are maintained in transmission cycles between tick vectors (*Ixodes* species) and vertebrate reservoir hosts. Different *Borrelia* genospecies vary in their vector/ host associations and human pathogenicity. However, the genetic basis for these adaptations is unresolved and requires complete genomes for comparative analyses. The genome of *Borrelia* is unusual for bacteria consisting of a linear chromosome and a large number of circular and linear plasmids. These plasmids are of particular interest as they carry genes involved in host and vector interactions, and are thought to underpin the adaptive processes responsible for species-specific phenotypes. However, assembling complete *Borrelia* plasmids remains challenging due to the high levels of genome complexity and intra-species plasmid homology (also known as mosaic structure). The recent development of high-fidelity (HiFi) SMRT PacBio long read sequencing, which promises >99% base calling accuracy, is a significant advancement in long-read sequencing technologies and could help resolve the assembly of complete *Borrelia* plasmids once and for all.

Methods

We generated and compared Illumina and PacBio (traditional and HiFi) data for 29 *Borrelia* isolates and used hybridSPAdes for Illumina/ PacBio read assembly and three different assemblers for PacBio data assembly (Microbial, improved phase (IPA), HiCanu). The PacBio Microbial assembler was used for the processing of traditional PacBio reads, the PacBio IPA assembler and the HiCanu assembler for PacBio HiFi reads. In order to reconstruct the genomes including complete plasmids and to compare the assemblies, quality control and contig refining steps were conducted including overlap analyses, detection of incomplete contigs, duplicates and missamblies and definition of plasmid type.

Results

Our results show that none of the sequence data and assembly strategies unequivocally performed best for the entire dataset and not even for all genome elements of one isolate. We observed a trend of low contig numbers resulting from the IPA HiFi data assembly but incomplete plasmids. In contrast, the HiCanu HiFi data assembly showed the highest number of contigs (due to duplicates) but many complete plasmids. No tendencies were observed based on the microbial traditional PacBio data assembly that was combined with the Illumina data. Depending on assembler and isolate, plasmids were incomplete or not recovered from one assembler but were probably (completely) reconstructed using another assembly strategy. Therefore, comparing and combining the assembly results lead to reconstruction of the completed genomes.
Conclusion

*Borrelia* plasmid reconstruction is still highly complex despite the latest available sequencing technologies and assembly methods. Although the PacBio HiFi data is quite promising, it still needs careful contig refining steps. Only by comparing, refining and combining the results of the different assembly strategies for each isolate, we were able to reconstruct completed genomes including all plasmids.
Introduction
Ticks can be found in a wide range of habitats where suitable hosts and suitable places for the often extended off-host phases are present. Cities and their environs offer a multitude of living opportunities for ticks and their hosts such as neglected buildings, parks, gardens, cemeteries, fallow land, and often also forests. Some tick species also act as vectors of pathogens. Human Lyme borreliosis, for instance, is a health problem in Berlin with 700-1000 reported cases per year among the approximately 3.7 million inhabitants.

Methods
Published and as yet unpublished records, spanning a period of nearly 100 years, of ticks collected in and around the metropolitan area of Berlin were used to create a high-resolution city map. Both endemic tick species and occasional findings of non-endemic tick species were considered.

Results
There are 237 reliable records of two argasid and 10 ixodid species: Argas reflexus, Carios vespertilionis, Ixodes ricinus/inopinatus, Ixodes hexagonus, Ixodes canisuga, Ixodes trianguliceps, Ixodes arboricola, Ixodes frontalis, Dermacentor reticulatus, Haemaphysalis concinna, Hyalomma rufipes, and Rhipicephalus sanguineus s.l. The most abundant tick species are I. ricinus/inopinatus and D. reticulatus. The mentioned ticks strongly vary as to their biology (e.g., life cycle, environmental preferences etc.). Among them are endophilous/nidicolous and exophilous species, catholic feeders and others specialized to parasitize a certain group of hosts. Some of the 12 tick species are known to bite humans, especially I. ricinus/inopinatus, A. reflexus, Ha. concinna, and Hy. rufipes. Some such as D. reticulatus have been emergent during the past 2–3 decades, whereas the abundance of other species such as A. reflexus have decreased during this period. Hyalomma rufipes, and R. sanguineus s.l. have been exotic visitors, but provided that temperatures continue to rise in the future, they may have some chance to establish themselves in this area. In addition, the occurrences of Ixodes rugicollis, newly described from pine martens by Schulze and Schlottke in 1929, and Ixodes vespertilionis have been documented in or close to Berlin, but the exact locations where they were found are unfortunately not known.

Conclusion
At least 14 tick species are established in or around Berlin or at least occur there sporadically. Some of the listed species bite people and may transmit pathogens, other species live a rather hidden life. Quite a number of vertebrates (e.g. pigeons, foxes) have reached a very high abundance in and in the environs of Berlin, and ticks may take advantage from the high availability of certain host species there. The circumstances of the occurrence of some selected tick species in Berlin and their (sub)urban ecology will be further highlighted in the presentation.
Introduction

Anaplasma phagocytophilum (Ap) is the causative agent of human granulocytic anaplasmosis transmitted by the blacklegged tick (Ixodes scapularis) in the eastern USA. Traditionally blood has been the tissue used to screen for Ap since it is a blood borne pathogen infecting neutrophils.

Aims

The main objectives of this study were to compare Ap-infection results based on host blood and biopsy samples and to quantify the levels of Ap infection in mammalian hosts in a north central USA field site where Ap is endemic.

Methods

Small to medium-sized mammals were trapped using pitfall, Sherman and Tomahawk traps in Fort McCoy, Wisconsin from 2010 - 2012. DNA from host blood samples and biopsies were screened for Ap by a qPCR targeting the msp2 gene. Positive samples were confirmed using a nested PCR targeting the 16S rRNA gene.

Results

A total of 2255 capture events comprising 14 mammal species were captured. In total, 1210 biopsy samples and 515 blood samples were assayed. Both blood and biopsy samples were collected from 421 individuals which comprised 7 species: Didelphis virginiana, Myodes gapperi, Peromyscus leucopus, Procyon lotor, Tamias striatus, Urocyon cinereoargenteus, and Zapus hudsonius. For the individuals that had both biopsy and blood samples, we found the total host infection prevalence of Ap based upon biopsy samples was 24.9%, while based upon blood samples, it was 29.0%. A closer look at biopsy and blood samples showed that 13.5% was Ap positive by both blood and biopsy, 11.2% by biopsy only and 15.2% by blood only. For the individuals that had both biopsy and blood samples, we compared the Ap infection prevalence from biopsy and blood for each species using a Fisher’s exact test with a Bonferroni correction applied. In P. leucopus there was a significantly higher Ap infection prevalence in blood samples (biopsy only 10.4%, blood only 14.8%, p-value=0.0005); for all other species, there was no significant difference between sample type.

Conclusion

In many Ap infection prevalence studies conducted on wildlife in the US, blood samples are the primary type of tissue collected. Collecting biopsy samples in the field may be an easier option compared to blood. For samples where Ap was detected in biopsy but not by blood, it may be because Ap is short-lived in the blood. This presence of Ap DNA in biopsy samples may be because Ap is short-lived in the peripheral bloodstream in contrast with other tissues like the spleen and ear tissues. To best compare the PCR results between biopsy and blood samples, a laboratory transmission study should be conducted over the course of the infection. Furthermore,
incorporating xenodiagnoses at each sampling time point would help interpret the biological meaning of the biopsy and blood sample infection results.
Habitat suitability modeling of the blacklegged tick, *Ixodes scapularis*, in Michigan, USA, an active area of invasion

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Introduction
In the eastern USA, the blacklegged tick (*Ixodes scapularis*) is the principal vector of several pathogens including those that cause Lyme disease and human granulocytic anaplasmosis. In Michigan, blacklegged ticks were first discovered in the late 1980s and are still spreading across the state. Being able to predict the spatial distribution of disease risk is important for targeting public health prevention messages and for diagnosing and treatment of disease.

Aims
The objective of this study was to develop habitat suitability models using maximum entropy (MaxEnt) to predict suitable habitats for the blacklegged tick in Michigan.

Methods
Blacklegged ticks were drag sampled throughout Michigan from April - November, 2017 - 2021, targeting the adult and nymphal activity periods. At least 1000 m² were drag sampled per site per visit. The MaxEnt approach, which only considers presence data, was used to model suitable habitats in Michigan. According to the CDC, a site is classified as having an established tick population if at least six ticks of one life stage or two ticks of two different life stages are detected within a calendar year. Otherwise, a site is classified as “reported” if at least one blacklegged tick were found. Based on CDC criteria, we compared outputs for three models where tick presence comprised 1) all sites with at least one detected blacklegged tick from any year (i.e., least strict and most inclusive model, n=171); 2) all sites with established populations based on at least one of the establishment criteria (intermediate model, n=117); or 3) all sites where both establishment criteria were attained (i.e., most strict and least inclusive model, n=87). Twenty-nine environmental variables were initially used. A random subset (50%) of the positive sites was used to train each model, and then the remaining sites were used to test the model predictions.

Results
The area under the curve (AUC) values were 0.938, 0.946 and 0.968 for the least, intermediate, and strict models, respectively. In all models the same five critical environmental variables were identified as important for developing the model: amount of leaf litter, percent tree canopy cover, landcover features, average snow density and growing degree days. According to the models, the most suitable habitats were found along coastal and southern Michigan. Conversely, the lower probability occurrence sites were found in the northern areas in the Lower Peninsula and eastern areas in the Upper Peninsula.

Conclusions
The habitat suitability models based the three levels of tick presence criteria examined produced very similar results. Previous studies also have shown southern Michigan in the Lower Peninsula and the western portion of the Upper Peninsula have suitable sites. Coastal sites and southern sites might be conducive for ticks because of the climate supporting tick survival and development.
This work aims to determine whether *Borrelia burgdorferi* sensu lato occurs in the populations of green lizards (*Lacerta viridis*) and ticks in lizard habitats in the Czech Republic. Green lizards are common hosts of tick larvae and nymphs and might be involved into the transmission cycle of *Borrelia burgdorferi* s.l. The lizards (Lacertidae) are potential reservoir for *Borrelia lusitaniae* and this species of *Borrelia* was reported from around the Mediterranean basin and also from central and northern Europe (Slovakia, Poland, Latvia).

Green lizards were sampled in two areas at the Tiché Údolí Nature Reserve (site A: 50.1482N, 14.3669E; site B: 50.1476N, 14.3745E), Central Bohemian Region, Czech Republic. The skin biopsy specimens were collected from 52 captured lizards. In total 488 engorged ticks (374 larvae, 114 nymphs) were removed from 30 out of 52 captured green lizards. Also, 17 questing ticks from both areas (site A 90, site B 83) were collected by flagging. DNA was isolated from skin biopsy and whole ticks and all the 713 samples were examined for presence of *B. burgdorferi* s.l. by amplifying fragment of the rrflA—rrfB intergenic spacer. All positive samples were identified by direct sequence analysis.

The touchdown polymerase chain reaction and gel electrophoresis revealed *Borrelia lusitaniae* in three lizard tissue samples. Most lizards (19/30, 63%) had at least one *Borrelia* positive tick. *B. lusitaniae* formed 92% (34/37) and 59% (17/29) of all borreliae detected in larvae and nymphs, respectively. *B. lusitaniae* (8/12, 67%) was also the major pathogen in the questing ticks from site B. At site A, 14% (3/21) of ticks were positive for *B. lusitaniae*.

It can be concluded that *B. lusitaniae* is a common pathogen at lizard sites in the Czech Republic. As lizards often inhabit urban areas, the data presented may also contribute to raising awareness of the spread and risk of *Borrelia* infection.
P014 - Molecular investigation of Borrelia Relapsing Fever and Rickettsia spp. in ticks from different regions of Morocco

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Introduction
Tick-borne pathogens are an important cause of diseases in human and animal populations. Identifying which pathogens are circulating in specific geographic areas is crucial to understand the epidemiology of those diseases.

Methods
In this study, ticks were collected from animals and vegetation in nine regions with 13 sampling sites of Morocco during 2018-2021. Ticks were identified by morphological characters and DNA was extracted. Tick species were confirmed by PCR targeting a partial region of the 16S rDNA and screening for pathogens was performed for Borrelia burgdorferi s.l., Relapsing Fever Borrelia and Rickettsia spp. using specific sets of primers targeting different genes.

Results
A total of 47 ixodid ticks, adults (n=45) and nymphs (n=2), were collected from animals (n=30) and from the vegetation (n=17). Tick species were identified as Hyalomma aegyptium (n=20), H. anatolicum (n=13), H. dromedarii (n=3), H. impeltatum (n=4), and R. sanguineus (n=7). The overall prevalence of tick-borne pathogens in tick samples was 17.8% (8/45). Five were positive for Relapsing Fever Borrelia, five were positive for Rickettsia spp. and two ticks were co-infected. Sequencing results revealed the presence of B. turcica in the five positive H. aegyptium ticks collected from Testudo graeca. R. massiliae (n=1) was detected in one R. sanguineus from an hedgehog, R. sibirica mongolitimonae (n=4) was detected in three H. aegyptium collected from T. graeca and one H. anatolicum from vegetation. Two H. aegyptium removed from T. graeca were coinfectd with B. turcica and R. sibirica mongolitimonae.

Conclusion
To the best of our knowledge, this is the first detection of Borrelia turcica for Western North Africa.
P015 - Plasmid diversity and host association in Borrelia

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Introduction
Borrelia burgdorferi sensu lato forms a species complex of spirochetal bacteria that are maintained in natural transmission cycles by tick species of the genus Ixodes and small to medium sized vertebrate hosts. Phylogenetic investigations of chromosomal loci have shown that ecologically divergent strains form clusters that correspond to species. The species occupy different ecological niches as reflected in their host- and vector-associations. These associations of Borrelia species have been recurrent in the evolutionary history of the species complex. The genome of Borrelia species is unusually complex in that it comprises a linear chromosome and a number of linear and circular plasmids. Plasmid located genes and their products are known to play a major role in host- and vector-interaction of Borrelia. Initially shown for Borrelia burgdorferi sensu stricto in North America, plasmid types (defined by PFam32 loci) present in strains may vary even within a single species. Here we show that this is a general pattern in Borrelia as this was found in B. bavariensis and B. garinii, two of the species common in Europe and Asia and frequently involved in human Lyme borreliosis.

Methods
We use next generation sequencing techniques (Illumina, Pacific Biosciences) for reconstruction of Borrelia genomes. De novo assembly and read mapping to reference genomes were conducted in SPAdes, CLC genomics workbench, and HGAP. Plasmid types were defined by PFam32 or related loci (PFam49, PFam 52, PFam57/62).

Results
As plasmids are highly dynamic, complete reconstruction in Borrelia was not possible using Illumina reads only in combination with read mapping. Plasmid dynamics was even observed in genetically homogeneous populations such as European B. bavariensis. Contigs that do not have PFam32 or related genes cannot be “officially” associated to a plasmid type as gene content does not relate to plasmid type. Using examples of Borrelia species adapted to various reservoir hosts (birds, rodents) and tick vector species (Ixodes persulcatus, Ixodes ricinus) we demonstrate that i) plasmid numbers vary between and within species, ii) fusion plasmids are formed. We show using examples of B. bavariensis (rodent-adapted) and B. garinii (bird-adapted) that plasmid variation is difficult to be associated with reservoir hosts. Gene differences between Asian and European B. bavariensis populations may provide interesting candidate genes for unravelling adaptation to the tick vector (Asia: Ixodes persulcatus; Europe: Ixodes ricinus).

Conclusion
Plasmid reconstruction is challenging in Borrelia as these are highly dynamic, not only between but also within species, even in genetically homogeneous populations such as European B. bavariensis. Short read technologies are insufficient for reconstruction of completed Borrelia genomes and strategies for obtaining completed genomes need careful design. Further work is
needed to elucidate the reasons for the observed plasmid dynamics and how this relates to human pathogenicity.
**P016 - OspE mediates Borrelia burgdorferi strain-specific complement evasion in Eastern fence lizards**

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**Introduction**
Lyme disease is primarily caused by *Borrelia burgdorferi* sensu stricto (*Bb*) in North America, transmitted by *Ixodid* ticks between multiple vertebrate hosts, and serves as an archetypal model for arthropod-pathogen-host interactions. While *Bb* is frequently observed in mammalian and avian reservoirs, the bacterium is rarely isolated from North American reptiles. Two closely related lizard species, the Eastern (*Sceloporus undulatus*) and Western (*Sceloporus occidentalis*) fence lizards, can be found parasitized by ticks in Lyme endemic areas, but cannot harbor *Bb* infection. Vertebrates are known to generate complement as an innate defense mechanism that can be activated before *Bb* disseminate to distal tissues. In previous studies, complement from Western fence lizards has proven lethal against one *Bb* strain, which implies complement’s role in denying lizards as competent hosts. However, *Bb* DNA has been identified in distal tissues of field-collected Eastern fence lizards, suggesting that some *Bb* genotypes may overcome lizard complement-mediated clearance.

**Aims**

**Hypothesis:** *Borrelia burgdorferi* produce allelically-variable anti-complement proteins that promote strain specific lizard complement evasion

**Objectives:** Define the ability of diverse *Bb* genotypes to escape lizard complement mediated killing and identify *Bb* outer surface lipoproteins that confer complement evasion activity

**Methods**
We examined tails and sera from 40 field-collected Eastern fence lizards for sero-reactivity to, and presence of, *Bb*. We determined the ability of genotypically-distinct *Bb* strains to evade lizard complement by evaluating their ability to survive in sera (serum resistance). We also produced two *Bb* polymorphic ant-complement proteins, CspZ and OspE, in a serum sensitive *Borrelia* strains, and determined lizard serum resistance activity.

**Results**
We found that only one adult lizard produced seropositive results for *Bb* C6 competitive ELISA, aligning with low confidence PCR results from tail tissues. We showed that *Bb* displays strain variability in lizard serum resistance, that two *ospC* genotype-K strains survive in lizard sera at significantly greater levels than the background strains. We also identified OspE from this *Bb* genotype as a determinant for facilitating resistance to lizard serum.

**Conclusion:**
Results from wild-caught lizards suggests that some *Bb* strains may escape innate immune responses, surviving long enough to disseminate to distal tissues. Our findings on *Bb* strain-specific OspE-mediated serum resistance activity provides insight into the role of complement to determine *Bb* survival in lizards.
Ixodes ricinus ticks are considered as the vector of the Borrelia burgdorferi sensu lato complex in urban areas, including city parks and green recreational areas. The aim of the present study was to determine the prevalence of B. burgdorferi s. l. in urban areas in the city of Prague, Czech Republic. In selected public green areas in Prague, a total of 2819 I. ricinus ticks were collected in spring, from April to June, in 2014-2020. Quantitative real time PCR targeted a gene fragment of outer surface protein A (ospA) revealed 28.1% of ticks (31% of males, 33.7% of females and 25.8% of nymphs) to be positive for B. burgdorferi s. l. The prevalence varied significantly (p<0.01) between collection sites, with the highest numbers of infected ticks found in the central city areas. The places serving people for recreational and sport activities in urban areas are characterized by a lower diversity of reservoir hosts, provide opportunity for exposure to Borrelia infected ticks, and pose a higher infection risk. We have detected seven Borrelia species in ticks: B. garinii, B. afzelii, B. bavariensis, B. burgdorferi sensu stricto, B. valaisiana, B. spielmanii, and B. finlandensis. Most positive ticks were infected by B. garinii (35%) and B. afzelii (36.9%). Our results show that the Borrelia transmission cycle occurs within urban biotops and highlight the need for surveillance of tick-borne pathogens in public green areas.

Acknowledgements:
This research was supported by MH CZ-DRO (The National Institute of Public Health - NIPH, 75010330).
**P018 - Detection of Emerging Tick-Borne Disease Agents in The French Riviera**

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**Introduction**
Lyme borreliosis is a zoonotic tick-borne infection representing the most frequent vector-borne disease in the northern hemisphere. The Mediterranean rim is generally described as unsuitable for the European vector, *Ixodes ricinus*. Thus, southeastern is considered non endemic for Lyme Borreliosis by local clinicians.

**Objections/Aims**
We conducted an epidemiological study to assess whether *I. ricinus* was present and study its infection status for tick-borne bacteria.

**Methods**
Ticks originating from southeastern France were obtained from flagging sampling and removed from animals and tick-bitten patients. Species level identification used morphological keys and Matrix Associated Laser Desorption Ionization-Time Of Flight Mass Spectrometry. Quantitative PCR and sequencing assays were used to detect and identify tick-associated bacteria (*Borrelia*, *Rickettsia*, Anaplasmataceae, *Bartonella*, *Coxiella burnetii*) in each specimen.

**Results**
A total of 1232 ticks were collected in several localities. Among these, 863 were identified as *I. ricinus* (70%). Bacterial screening allowed identification of Lyme group *Borrelia* among *I. ricinus* ticks originating from various regional areas. Other emerging tick-borne pathogens like *Borrelia miyamotoi* and *Rickettsia* species were also detected.

**Conclusions**
The Alpes-Maritimes region, part of the French Riviera, harbours *I. ricinus* ticks infected with Lyme group *Borrelia* and several other tick-borne bacterial agents. Clinicians and outdoor activity participants should be aware of the local Lyme borreliosis transmission risk in this highly touristic area of Southeastern France.
P019 - Seroepidemiological survey among wild birds in Bulgaria for presence of specific antibodies against B. burgdorferi s.l.

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Introduction
Lyme borreliosis (LB) is the most common tick-borne human infection in Bulgaria with several hundred new cases a year. It is known that wild bird species, which are common for the urban environment are host for ticks from genus *Ixodes* and may be involved in natural enzootic transmission cycle of LB. Every year, regardless of active anti-tick measures, a large number of tick bites are recorded in urban areas of the country. There is no actual data on spreading of *B. burgdorferi s.l.* among wild birds and their importance in transmission of LB in Bulgaria.

Aim
To investigate seroprevalence of *Borrelia burgdorferi* s.l. in passerine birds from the most densely populated and large city of the country - Sofia.

Methods
Serum samples (n=118) from 10 bird species collected in the period June - October 2021 were tested by ELISA for specific IgG antibodies against *B. burgdorferi* s.l.

Results
In this study, specific antibodies against *B. burgdorferi* s.l. were detected in 32.2% (38/118) of all tested birds. Analysis of the results shows that out of 10 different bird species, antibodies were found in five of them - *Cyanistes caerulens*, *Garrulus glandarius*, *Parus major*, *Sitta europaea* and *Turdus merula*. The highest seroprevalence was observed in *P. major* - 42.4% (25/59) and *T. merula* - 33.3% (9/27). A significant difference was found in seroprevalence in the samples collected in early summer and in autumn. The seropositivity levels rise almost twice from 22.4% (11/49) in June-July to 39.1% (27/69) in September-October.

Conclusion
Our results observed prevalence of specific antibodies against the causative agent of Lyme borreliosis in wild passerine birds in Bulgaria. We established increased seroprevalence rates in the bird population after the months of tick’s activity. This confirms active circulation of *B. burgdorferi s.l.* between birds and the competent tick vector and emphasizes their significant role in transmission of LB. The highest levels of seroprevalence in *P. major* and *T. merula* define both species as a considerable reservoir for *B. burgdorferi* s.l. The study is focused over urban bird population, because of higher incidence of tick bites in humans and subsequent risk of transmission of LB. Data can contribute for better understanding of transmission, distribution and maintenance of LB in the country.
Introduction
Lyme disease is the most prevalent vector-borne disease in North America with an estimated 300,000 cases each year. Over the past few decades, blacklegged ticks (*Ixodes scapularis*), the primary vector of *Borrelia burgdorferi* sensu stricto in North America, have been invading the state of Michigan, USA. Ticks have been detected in more than half of the counties, several of which are endemic for Lyme disease. There are still several counties, particularly in the central region, in which blacklegged ticks have yet to be detected. Blacklegged ticks were first detected in Michigan in the Upper Peninsula in the 1980s, followed by in the southwestern Lower Peninsula in the early 2000s. Currently, blacklegged ticks are spreading northward and inland across the state. In 2019, 22% of Michigan counties contained blacklegged ticks that tested positive for *B. burgdorferi*. Here we aimed to update our understanding of the spread of blacklegged ticks and *B. burgdorferi* infection prevalence in Michigan, which is critical information for understanding Lyme disease risk in Michigan. These samples will also help us determine how *B. burgdorferi* diversity varies across a gradient of invasion for the blacklegged tick and how it changes within a site over the course of invasion. Because certain strain types have been associated with different clinical manifestations, it is important to understand the genetic composition of pathogen populations and infer the factors that underly their distribution over space and time.

Methods
We collected all life stages and species of questing ticks from sites in May-July from 2021 using drag sampling. We sampled at least two sites per county to increase our chance of detecting blacklegged ticks. We targeted sites on public land with suitable habitat for blacklegged ticks. We dragged 1600 m per site per visit, and drag cloths were checked every 20 m for ticks, which were stored in 95% ethanol. We determined species and life stage for each tick, extracted genomic DNA, and identified positive *B. burgdorferi* infections using a real time PCR targeting the 16S rRNA.

Results
In 2021 we collected 2588 nymphal and 539 adult *I. scapularis* across 142 sites in Michigan spanning several decades of initial blacklegged tick detection. County-wide infection prevalence and an updated map of the distribution of *B. burgdorferi* in Michigan will be presented.

Conclusion
Blacklegged ticks and Lyme disease risk have been expanding in Michigan since the late 1980s. Because of the dynamic nature, thorough surveillance for emerging ticks, *B. burgdorferi*, and other *I. scapularis*-borne pathogens informs on disease risk to humans and companion animals; this is crucial for the prevention, diagnosis, and treatment of tick-borne diseases. A future assessment of the population genetics of *B. burgdorferi* across endemic and invasion gradients will help characterize population structure over the course of establishment, which will also contribute to the understanding of the evolution and epidemiology of Lyme disease and other tick-borne diseases.
Male mice have a higher abundance of Borrelia burgdorferi in their tissues and infect more feeding Ixodes scapularis ticks compared to female mice

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Introduction
Host sex is an important factor in host-pathogen interactions and in the ecology of infectious disease. Male hosts are often more susceptible to infectious diseases and have higher pathogen burdens compared to female hosts. The tick-borne spirochete Borrelia burgdorferi sensu stricto (Bb) causes Lyme borreliosis in North America and establishes chronic infections in rodent reservoir hosts.

Methods
We recently conducted a controlled experimental infection study where male and female mice (Mus musculus, C3H/HeJ) were experimentally infected via tick bite with one of 11 strains of Bb (4 males and 4 females per strain). To determine host-to-tick transmission, mice were infested with Ixodes scapularis larvae at days 30, 60, and 90 post-infection (PI). The mice were euthanized at 97 days PI and 7 organs were dissected (bladder, heart, kidney, left ear, right ear, tibiotarsal joint, and ventral skin). All organs and xenodiagnostic ticks were tested for the presence and abundance of Bb using qPCR.

Result
Across the 11 strains of Bb, the percentage of infected organs in the male mice (65.4%) was significantly higher compared to the female mice (50.5%). Across the subset of infected organs, the abundance of Bb was 1.5x higher in the male mice compared to the female mice. Remarkably, the abundance of Bb in the ventral skin was 15.3x higher in male mice compared to female mice. Host-to-tick transmission of Bb was similar between males and females on day 30 PI (94.8% vs 97.3%) but it was higher for males compared to females on day 90 PI (82.6% vs 67.2%).

Conclusions
Our study suggests that in nature, male rodents are more important than female rodents for the transmission of this important tick-borne pathogen.
The evaluation of host antibody response to *Ixodes ricinus* as an indicator of exposure and disease risk

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**Introduction**

Ticks introduce a variety of salivary proteins into their host during feeding to successfully obtain a blood meal. Tick-transmitted pathogens are transferred to the hosts during feeding, and the actions of tick salivary proteins are essential for both tick feeding and pathogen transmission. Following repeated tick exposure, hosts may develop an immune response to tick salivary proteins. To better understand the epidemiology, pathogenesis, immunology, and clinical manifestations of the host tick bite response, quantitative biologic markers of tick exposure are required. One such marker may be host antibody directed against tick antigen.

**Methods**

In a systematic review exercise, we were able to select four tick salivary proteins for further investigation in the assessment of tick exposure, highlighting calreticulin as the most promising marker for anti-tick bite antibodies. In this work, we tested antibody responses to recombinant *Ixodes ricinus* calreticulin (rCRT) using a set of bovine serum samples obtained from cattle housed in different conditions; (a) raised outdoor and *Babesia divergens*-infected, (b) cattle that were also raised outdoor and *B. divergens* free, and (c) cattle that were raised indoors and therefore not likely to be exposed to ticks, thus acting as a putative negative control group. For the analysis of bovine sera antibody responses, an indirect enzyme-linked immunosorbent assay (ELISA) format was employed.

**Results**

We observed no significant difference when comparing IgG levels to rCRT between all groups of cattle serum samples. However, significant differences were observed between all 3 groups of cattle serum samples when comparing IgM levels. When comparing anti-rCRT IgG and IgM levels, we observed a weak positive correlation only in outdoor raised *B. divergens* positive cattle serum samples.

**Conclusion**

While tick saliva elicited antibody response has previously been demonstrated to be a marker for exposure in humans, The pattern of IgG response to *Ixodes ricinus* calreticulin in naturally challenged cattle was not consistent with exposure status, unlike the responses that have been observed in humans subject to tick bite, One hypothesis that explains this result is that the cattle hosts are producing cross reactive antibodies as a result of calreticulin exposure from other arthropod blood feeders. We are presently investigating this by assessing cross reactions to mite extracts (*Psoroptes* spp.) and the identification of *Ixodes* sp specific epitopes in calreticulin.
P024 - Lyme Borreliosis incidence in relation to mammalian abundance, climate, and landscape characteristics in Northern Europe

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Introduction
Circulation of tick-borne pathogens is determined by ticks, the availability of hosts that feed ticks and hosts that are reservoirs for the pathogens, as well as habitat characteristics and climate. The most common tick-borne disease in the Northern hemisphere is Lyme borreliosis (LB), caused by Borrelia burgdorferi sensu lato bacteria transmitted by Ixodes spp. ticks. However, the roles of different host species, climate and landscape characteristics in LB epidemiology have remained unclear, especially in the northernmost zone of the distribution of the tick vectors and the pathogen.

Aims
Here, we assess whether abundances of (1) B. burgdorferi s. l. reservoir hosts (voles and squirrels), (2) alternative hosts for immature or adult ticks (raccoon dogs, red foxes, and hares), (3) adult tick main reproductive hosts (moose and deer), (4) landscape characteristics, or (5) climate drive occurrence and incidence of LB in the human population.

Methods
We use long-term data on laboratory-diagnosed LB (1995-2018) and clinically-diagnosed LB (2011-2018) in Finland with dynamic species distribution models. To take into account the possibility that the drivers of human LB incidence variation differ in different regions, we divided Finland to three regions based on biogeographical areas: Northwest, Southwest and Southeast Finland.

Results
We show that LB risk (occurrence and incidence) was associated with different drivers depending on the biogeographical region within the country. Deer and moose abundance increase LB risk in Northwest Finland, but not in Southwest or Southeast Finland, where especially deer is more abundant. The results also suggest that abundances of raccoon dogs, red foxes and hares explain LB risk especially in Southeast Finland, but the direction of the effect was not consistent. Also, our results indicate that the habitat fragmentation increases LB risk. Moreover, LB risk is positively associated with the length of growing season in the entire study area.

Conclusion
To conclude, our study suggests that the factors explaining LB epidemiology are not the same everywhere, but vary spatially, depending on local climate, landscape and host community.
P026 - Unravelling the Intraspecies Diversity of Anaplasma phagocytophilum (AP)

Mr Sean Brierley

Introduction
AP is a tick-bone, intracellular parasite of the neutrophils, and the causative agent of granulocytic anaplasmosis (GA), a socioeconomically important immunosuppressive disorder of humans and livestock. Symptoms vary from subclinical to fatal and thus, are often missed or misdiagnosed. GA is now the second most common tick-bone disease (TBD) in North America and the most wide-spread TBD in Europe. Despite this established burden, the ecology and epidemiology of AP strains and the infections they cause remains unclear. AP epidemiological cycles involve several ecotypes, vectors and hosts, with significant variation between Europe and the USA. AP strain diversity has largely been quantified based on multi-locus sequence typing (MLST) which has identified 520 isolates and 311 sequence types, as well as variation in the groEL operon, which has grouped isolates into four ecotypes. Most AP strains fall within ecotypes I and II, comprised of strains that parasitise all known vertebrate hosts, except for rodents and birds, which exclusively reside in ecotypes III and IV respectively.

Aims
- Generate ultra-sensitive AP strain fingerprints from culture.
- Elucidate AP genetic diversity, host specificity, and epidemiology.

Methods
The Illumina MiSeq and Oxford Nanopore MinION were used to generate short and long read sequence data for AP strains grown in Ixodid tick-cell lines at the Tick Cell Biobank, Liverpool. Data from each system was combined into a consensus assembly with Unicycler and analysed with a series of contemporary bioinformatical techniques. Our data was investigated alongside all available AP whole genome data for comparative purposes.

Results
The chromosomes of five previously unexplored AP strains were successfully assembled from culture. Through comparative analysis, we have identified previously unrecognized delineations within ecotypes I and II of AP through the concatenation of 129 shared single copy genes.

Discussion
The concatenation of shared single copy genes from whole genome data has demonstrated not only the ability to further delineate ecotypes I and II through the separation of old and new world strains, but also accurately fingerprint AP strains from incomplete genomic data. Therefore, as AP whole genome data accumulates, a more accurate picture of strain epidemiology can be developed with increasingly more refined ecotypes, a step essential to the generation of future infection risk maps.
**Introduction**

Lyme borreliosis (LB) is the most common tick-borne disease in Europe. Vaccines for prevention of LB are in development. Understanding the LB disease burden will inform future vaccine implementation strategies by identifying geographic areas where incidence is highest, or risk is increasing.

**Methods**

We searched websites of government public health agencies and institutes for available national-level surveillance data reporting cases and/or incidence of LB in Europe from 2005-2020 (PROSPERO, CRD42021236906). When only LB cases were reported, age-specific incidence was calculated (in R Core Team (2021)) as the number of LB cases per 100,000 population per year (PPY) using available census data.

**Results**

Reports from public health surveillance systems were available for 26 countries in Europe. There was marked heterogeneity in surveillance systems (passive vs. mandatory vs. sentinel reporting), data sources (clinical and/or laboratory), case definitions, and LB testing methods, limiting data comparability. Three countries (Belgium, France, and Switzerland) employed sentinel surveillance systems whereas 23, but most surveillance systems (23 of 26 countries) were passive. Standardized case definitions such as those published in 2009 by the European Concerted Action on Lyme Borreliosis (EUCALB) were used in only two out of 26 surveillance systems.

The highest LB incidence (>100 cases per 100,000 PPY) areas occurred in Estonia, Lithuania, Slovenia, and Switzerland. Incidence of 40-80 per 100,000 PPY occurred in Croatia, France, Poland; 20-40 per 100,000 PPY per year in Finland, Latvia; and <20 per 100,000 PPY in Belgium, Bulgaria, Croatia, Hungary, Ireland, Norway, Portugal, Romania, Russia, Scotland, Serbia, and the United Kingdom (UK). However, high incidence was also observed at the sub-national level in France, Poland, the Czech Republic, and Belgium (all had local areas where the incidence was >100,000 PPY) and Germany (up to 80 per 100,000 PPY). There was no LB surveillance in most of Southern and Mediterranean Europe.

LB incidence in the Baltic States and Scandinavia increased in most recent years. Trends in the UK and Ireland were less pronounced and incidence rates appeared to be stable in much of Eastern Europe, with a decreasing trend in Romania. In some neighbouring
countries, LB incidence varied across national borders, reflecting differing surveillance approaches.

**Conclusion**
From 2005-2020, there was high variability in reported LB incidence across Europe, with the highest incidences occurring in the Baltic States and Slovenia. Wider implementation of common case definitions such as EUCALB and standardization of surveillance methodology are needed to interpret the differences in LB incidence observed across European countries.
Introduction
Lyme disease (LD) epidemiology has been characterized in North America, parts of Europe, and China; however, less is known about LD and *Borrelia burgdorferi sensu lato* (*Bbsl*) distribution in tick species beyond these regions.

Methods
We conducted a systematic literature review (SLR) from 2005-2020 to identify articles reporting burden of LD (incidence and seroprevalence) outside of North America, Europe, and China (PROSPERO: CRD42021236906). Publications with data on four main vector-competent *Ixodes* (*I.*) tick species (*I. pacificus*, *I. scapularis*, *I. ricinus*, and *I. persulcatus*), infected with ten clinically relevant genospecies of *Bbsl*, were also included. Additionally, websites of government public health agencies from countries in Asia, Africa, Americas, Eastern Mediterranean, and Russia were searched for surveillance reports on LD.

Data from SLR articles that met inclusion criteria were summarized, and public health surveillance data were analyzed. *Bbsl* infection rates (IRs) in vector competent tick species were evaluated from articles that reported infection with clinically relevant *Bbsl* genospecies. Incidence, and corresponding 95% confidence intervals (CIs), were calculated from surveillance data using available census data. Available data were organized by geographic clusters: Asia (Northeast, South), Americas (Central, Amazon), Eastern Mediterranean, and Russia.

Results
Incidence for LD (cases per 100,000 persons per year) was only available in Russia and (Northeast) Asia (Japan, and Korea). In Russia, incidence remained constant from 2009 to 2020, with a range of 2.86—6.96 (95% CI: 2.77—7.1). Incidence was lower in Japan and Korea, with mean incidence of 0.01 (95% CI: 0.006—0.017) in Japan; 0.032 (95% CI: 0.019—0.052) in Korea. Seroprevalence (antibodies to *Bbsl*) was obtained from 24 studies in ten countries: 4 in Asia (India, Malaysia, Mongolia, 8 in the Americas (Brazil, Colombia, Cuba, Mexico), 11 in the Eastern Mediterranean (Jordan, Turkey), and 1 in Russia. Although intra-country variation was considerable, LD seroprevalence in the general population ranged 0—19.9% in Asia (0—14% Northeast; 0—19.9% South); 0—29% in Americas (0—11.1% Amazon; 2.09—29% Central); 0-29% in Eastern Mediterranean; 10.3% in Russia. In ten studies, seroprevalence rates were higher in women (range: 1—15.6%) than in men (range: 0—13.9%).

Conclusion
LD seems to be present beyond North America, Europe, and China, though available evidence is limited to some countries in the regions of Asia, Americas, Eastern Mediterranean, and Russia.
Tick surveillance studies are required to identify areas where LD may be emerging; additional LD burden studies across the world are needed.
Introduction
Lyme borreliosis (LB) is the most common tick-borne disease in Europe; yet epidemiological data remain incomplete. Data from the published literature may be used to complement surveillance findings and strengthen our understanding of the burden of LB in Europe, supporting the implementation of preventative interventions including future vaccines.

Methods
We conducted a systematic literature review across PubMed, EMBASE, and CABI Direct (Global Health) databases (from January 1, 2005 to November 20, 2020) to identify studies reporting the incidence of LB in one or more European countries (PROSPERO protocol, CRD42021236906). Non-English articles were retained and translated in DeepL; data were extracted in DistillerSR.

Results
The systemic literature review yielded 65 unique papers describing LB incidence from one (or more) countries in Europe, providing at least one incidence estimate for 29 countries. There was substantial heterogeneity in study designs, populations sampled, and case definitions. The EUCALB (European Concerted Action on Lyme Borreliosis published in 2009) standardized LB case definitions were used by 13 (20%) of the 65 articles.

Of the 65 articles, 33 provided national-level LB incidence estimates. Among these, the highest LB incidence rates (>100 cases per 100,000 population per year [PPY]) were reported for Belgium, Finland, the Netherlands, and Switzerland. Incidence rates of 20-40 per 100,000 PPY were reported for Czech Republic, Poland, Germany. Incidence rates of <20 per 100,000 PPY were reported for all countries in the United Kingdom (UK), Republic of Ireland, Denmark, Sweden, Belarus, Russia, Slovak Republic, Portugal, and the Republic of Croatia.

Of the 65 articles, 49 provided incidence estimates at sub-national (regional) level. Higher incidence was observed at the sub-national level than at the national level in 8 countries, including the Republic of Ireland (up to 43 per 100,000 PPY), Scotland (up to 56.4 per 100,000 PPY), England (up to 23.4 per 100,000 PPY), Bulgaria (up to 30.9 per 100,000), Poland (up to 200.9 per 100,000 PPY), Russia (up to 40.5 per 100,000 PPY), Slovak Republic (up to 52.1 per 100,000 PPY), and Sweden (up to 464 per 100,000 PPY). Local studies conducted in Lithuania and Norway reported LB incidence of 85.4 and 552 per 100,000 PPY, respectively.

Conclusion
Reported LB incidence varied across Europe; furthermore, there was variability within countries, with high incidence rates reported in numerous areas in countries with an apparent low national incidence. Well-designed epidemiological studies that use standardized surveillance methods and case definitions are needed to better delineate incidence of LB within countries.
Introduction
Lyme borreliosis (LB) is the most common tick-borne disease in Europe, and seroprevalence data can be used to monitor endemic geographical regions, populations at higher risk of exposure and changes in disease risk over time. *Borrelia burgdorferi sensu lato* (*Bbsl*) seroprevalence among the general population, as well as those with frequent exposure to ticks, remains incompletely documented.

Methods
We conducted a systematic review from 2005-2020 of articles reporting LB seroprevalence (antibodies to *Bbsl*). Test results were summarized from studies that used single-tier testing; final test results were interpreted from algorithms of studies that used 2-tier testing (standard or modified).

Results
The search yielded 52 articles from 21 European countries. Most countries were represented by a single study except for Poland and Turkey (ten articles each). Most (45) studies applied cross-sectional design. The articles provided a mixed view of data that encompassed countries of known high and low endemicity, groups at differing risk of exposure to ticks, and varying antibody testing methods and strategies.

There were 33 population-based studies conducted among the general population (of these, 13 were nationally representative). The seroprevalence of LB varied substantially across European countries. In studies conducted among the general population, seroprevalence ranged from 2.7% in Norway to 20% in Finland. There was a general trend of higher seroprevalence in Eastern Europe and the Baltic States, compared to Southern and Western Europe. Intra-country variation was high.

Thirty (of 52) studies also reported seroprevalence among cohorts at higher risk of exposure. Seroprevalence was higher in persons undertaking at-risk occupational or leisure activities in some studies, but this was not consistently observed. Seroprevalence was as high as 72% (hunters aged 60-69 years in Austria) among some high-risk groups. Seroprevalence increased with age and was usually higher in men, except for Turkey where most studies showed a higher seroprevalence in women.

Diagnostic testing methods and strategies varied by studies. Of 52 studies, 25 used single-tier testing strategies, whereas 24 used standard 2-tier testing and three used modified 2-tier testing.
strategies. Three studies used the variable major protein [Vmp]-like sequence, expressed (VslE)-based enzyme immunoassay test.

**Conclusion**

In Europe, there was a wide variation in the seroprevalence of LB among the general population and persons with frequent exposure to ticks. High seroprevalence estimates identified among individual risk groups could help guide future public health interventions, such as vaccination.

Data interpretation and comparison across studies may be hampered by heterogeneity between studies. Harmonized approaches to serological testing, combined with more nationally representative seroprevalence studies, are needed to better understand LB exposure in Europe.
P031 - Assessing the ecological covariates related to tick-borne encephalitis emergence in Europe

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Introduction

Tick borne encephalitis (TBE) is a disease which can lead to severe neurological symptoms caused by the TBE virus (TBEv), a flavivirus transmitted in Europe mainly by ticks of the Ixodes complex. In nature, TBEv circulation is maintained in the environment by the co-occurrence of three major components: the virus, the vector and competent hosts, namely rodents. TBEv is typically found in hotspots (foci of infection) and so does not mirror the vector and host distributions. In recent decades, the incidence of TBE human cases in Europe has been rising both in endemic and new regions, with altitudinal and latitudinal shifts, posing an increasing threat to public health. Therefore, the early detection of new TBE foci represents a sanitary priority at community level.

Methods

We systematically reviewed the existing literature including data on covariates associated with the circulation of TBEv in Europe. We then validated our results by means of multiple linear regression, using as response variable TBE incidence (provided by the European Surveillance System: TESSy), averaged between 2017 and 2020, at the regional spatial level for ten different countries that represent the spatial variability of TBEv across the European continent. Explanatory variables, including climatic, environmental and ecological factors, were selected according to the literature review findings.

Results

We retrieved and analyzed data from sixty-three full text papers considering both biotic and abiotic factors. Our statistical findings highlight the predominant role of temperature-dependent variables, such as mean temperature in winter, autumnal cooling rate and mean diurnal temperature range, in explaining the variation of TBE incidence across European Countries, at the regional level. Variables linked to the ecology of the hosts and the vegetation cover, namely the probability of presence of competent hosts (Apodemus flavicollis, Myodes glareolus), of other suitable hosts (Cervus elaphus, Capreolus capreolus, Dama dama) and forest cover indexes (Enhanced Vegetation Index, % of forest areas), are also shown to play a significant role in TBEv circulation.

Conclusion

The existing literature is very heterogeneous, both in study design and variable types, and lacks information concerning thresholds of disease emergence. We therefore identified, summarized and
validated the covariates with the highest predictive power for TBE incidence at the regional spatial level and aimed to provide useful recommendations of consistent approaches for future work. Our results can support forthcoming modeling efforts to estimate the risk of TBEv infections and help decision-makers to identify emerging risk areas.

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Lyme disease (LD) is the most common vector-borne illness in the U.S. Borrelia burgdorferi (Bb), B. mayonii (Bmay) and B. miyamotoi (Bmiy) are causes. U.S. military service members in LD-endemic regions have unique training risks. We retrospectively surveyed exposure to Bb, Bmiy and Bmay while stationed at endemic areas. Paired pre-station and ≥ 180d sera were acquired from the Armed Forces Serum Repository from 920 active duty service members, between 18 and 74y; 460 in the Upper Midwest (UM) and 460 in the Northeast (NE). All were stationed for ≥3 months between 2006 and 2017. Sera were screened using a C6 IgG ELISA for Bb (and Bmay). Western blot was used to confirm Bb or to detect IgG to a Bmay 25-kDa antigen not identified by control Bb sera. IgG antibody screening for Bmiy used a modified ELISA GlpQ assay. Seropositive samples were qPCR tested for Bmay and Bmiy DNA. Of 920 pairs, 28 had C6 peptide IgG (61% NE, 39% UM). 21 had positive Bb IgG or IgM Western blots, and 10 were incident infections (5 UM, 5 NE). Overall Bb seroprevalence was 2.4 infections/100 persons over 11 years; incidence was 558/100,000 person-years. 3 subjects with positive C6 and negative Bb Western blots had IgG to a Bmay 25 kDa antigen, including 2 from the UM. No sera had Bmay DNA by qPCR. Of 920 subjects, 56 (6%) had GlpQ IgG antibodies, including 27 (3%) incident and 29 (3%) prevalent seropositives. Bmiy PCR on 53 samples from C6 positive pairs was negative in all but one subject with serologically confirmed LD. Estimated Bmiy seroprevalence was 2.9 infections/100 persons over 11 years, and incidence was 1483/100,000 person-years. Borrelia infections among military personnel in high incidence regions are at 9-115 fold higher risk for infection than civilians in the same areas.
P033 - Impacts of Alpha-gal Syndrome on Health-Related Quality of Life in Adults

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Introduction
Alpha-gal Syndrome is a rapidly emerging tick-borne disease that leads to mammalian meat and product allergies with symptoms varying from mild to life-threatening. A diagnosis of Alpha-gal Syndrome, especially as an adult, has the potential to negatively impact mental health and health-related quality of life. Prior research on both tick-borne diseases and food allergies have demonstrated significant mental health, well-being, and adjustment problems related to diagnosis and management.

Aim
The aim of this study was to explore Alpha-gal Syndrome as an adult-onset allergy and changes in health-related quality of life based on perceived severity of mammalian food and product allergy symptoms.

Methods
A mixed-method survey was conducted in 2021 with adults diagnosed with Alpha Gal or Mammalian Meat Allergy assessing their quality of life, including social and emotional functioning as measured by the Food Allergy Quality of Life Scale (FAQLQ-AF) and the Rand-36 Health-Related Quality of Life Survey. Snowball sampling was used to recruit participants through email and social media sites. Pearson correlations were conducted to assess relationships between factors of well-being and perceived severity of alpha-gal symptoms. A one-way Welch ANOVA was conducted to determine if the FAQLQ-AF scores were different for groups with different levels of perceived severity of alpha gal (1-5). Qualitative responses were hand-coded then analyzed for patterns, frequency and themes using NVivo and Quirkos.

Results
A total of 412 adults diagnosed with Alpha-gal Syndrome over the age of 18 participated. Quantitative analysis demonstrated a statistically significant relationship between perceived severity of alpha-gal symptoms and self-reported social functioning ($r(406) = -.26, p < .001$), physical functioning ($r(406) = -.20, p < .001$), and emotional role functioning ($r(403) = -.18, p < .001$). In addition, FAQLQ-AF scores were statistically significantly different between different severity groups, Welch's $F(4, 53.151) = 8.766, p < .001$. Qualitative analysis showed marked differences in how participants described their experiences and effects on quality of life based on their symptom severity rating, which ranged from mild with few to no reactions to severe, highly reactive and life threatening.

Conclusion
Findings suggest that as perceived symptom severity rating increases, emotional, social, and physical functioning decrease. A difference in quality of life based on perceived symptom severity was also shown. A patient symptom rating and health-related quality of life impact continuum may help both providers and patients better recognize and understand the complexity and variability of Alpha-gal Syndrome and its effects on quality of life. It is recommended that healthcare providers routinely assess the perceived severity of allergy symptoms and quality of life of all patients diagnosed with Alpha-gal Syndrome and provide them with evidence-based resources to help them adjust to this life-changing diagnosis.
P034 - Assessing the spatial distribution of ticks infected with tick-borne pathogens in The Netherlands

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Introduction

Tick-borne diseases are of increasing importance in The Netherlands, as the incidence of tick bites has tripled in the last twenty years. For disease prevention and control, knowledge on the risk areas for tick bites and tick infection with tick-born pathogens is essential. The objective of the present study was to explore the spatial occurrence of a wide variety of tickborne infections in the Netherlands with special interest to their relationship with urbanity.

Methods

From 2012 to 2015 the general public reported tick bite locations and ticks were collected after their removal from the skin through a web-based surveillance (Tekenradar.nl). Ticks were sent in and tested for a wide variety of tick-borne pathogens. The data were mapped and analyzed using additive regression models. Thus we compared the distribution of ticks infected with a specific pathogen compared to the general distribution of tick bite notifications, also in relation to urbanity.

Results

The distribution of tick bite reports showed a clear spatial clustering, indicating specific areas with a high tick bite density. The spatial distribution of the locations where tick bites were reported show great similarity to the spatial distribution of the tick bite consultations reported by GPs in previous studies, although the latter was relative to the population density. Preliminary analysis show a significant relation with urbanity for \textit{Neoehrlichia}, \textit{Anaplasma} and \textit{B. burgdorferi} s.l. For some of the other pathogens, including \textit{Rickettsia Helvatica}, spatial clustering was found. More results will be available for presentation during the conference.

Conclusion

Linking spatial data of tick bite and tick infection to environmental, climatic and recreational explanatory variables in future can be an important contribution to more precise risk mapping of tick bites and tick-borne infections. This may facilitate and guide future prevention and control efforts.
P035 - Population levels of exposure to Borrelia burgdorferi: seroprevalence studies in England, 2016-18 and 2021-22

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Introduction
Antibodies specific for Borrelia burgdorferi sensu lato (s.l.), the causative agent of Lyme disease (LD), can persist for several years after initial infection, allowing determination of seroprevalence as an estimate of population exposure to B. burgdorferi. A 2017 evidence review of the status of LD research in the UK has highlighted the lack of seroprevalence data for England.

Methods
The prevalence of antibodies specific to B. burgdorferi s.l. in the English population is being estimated in two retrospective cross-sectional cohorts selected from the following archives: 3,070 residual serum samples (2016-18) from routine microbiological testing and ~10,000 random blood donor plasma samples (2021-22), originally collected for SARS-CoV-2 serosurveillance. As a preliminary study, sera from 2016-18 were tested using a standard two-tiered testing (sTTT) strategy with the C6 Lyme ELISA (Immunetics) followed by the Borrelia ViraChip IgG test (Viramed Biotech AG) for any sera with positive or indeterminate reactivity in the ELISA. To test sera from 2021-22, the C6 Lyme ELISA will be replaced in the sTTT protocol by the B. burgdorferi VlsE/pepC10 IgG/IgM test system (Zeus Scientific).

Results
For the 2016-18 cohort, excluding residual diagnostic samples originally submitted for LD or syphilis testing, 2561/3070 samples had valid results in the C6 ELISA. 8.32% (95% CI 7.46 - 9.26) were C6 positive and 0.90% (95% CI 0.64 - 1.26) were IgG positive or indeterminate in the confirmatory assay. The 2016-18 cohort was not geographically representative of all of England with the majority of samples coming from the North West (49%) and South West (39%) regions with seroprevalences of 0.72% (95% CI: 0.38-1.36) and 1.22% (95% CI: 0.70-2.11) respectively after sTTT. Samples from the East of England (134/2561) had the highest C6 seropositivity (14.18%; 95% CI: 9.27 - 21.09), but none were reactive in the IgG Virachip assay.

Conclusions
Using a sTTT algorithm, a preliminary crude estimate of 0.90% for B. burgdorferi s.l. seroprevalence in England (2016-2018) was derived, with evidence for regional variations. Data from an ongoing more comprehensive study of ~10,000 normal blood donor samples (2021-22) analysed using an alternative tier 1 ELISA will test this estimate. Due to improved geographic
coverage and representation, this will allow geospatial analysis of population exposure to *B. burgdorferi* across the country.
P036 - TekenNet: Trends in tick bites in Belgium between 2016-2021 recorded via a citizen science platform

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Introduction
Since 2016, a citizen science platform has been in place in Belgium to report human tick bites. The aim of the TekenNet project is to map risk areas in the country based on the notifications made on the platform.

Methods
The notifications from 2016 to 2021 were considered for analysis. After cleaning the data, following variables were used for analyses: date of the tick bites and environment, location and activity during which the person was bitten.

Results
The geographical spread of tick bites was stable over the years, with a higher risk of being bitten by a tick in eastern Belgium. Across all years, tick bite reports generally started in March and ended in October, with a peak in reports in June. Tick bites occurred most often in the vicinity of people's house: 67.7% of the notifications originated within 5 km of the house, and 9% within 5 to 10 km. The garden was the most common place where people were bitten. Most bites (88.5% of notifications) happened during leisure activities. The results of TekenNet represents the behavior of humans as well as the presence and activities of ticks. A reduced amount of tick bite notifications during the active tick season could often be correlated with meteorological factors: when it was cold and rainy, people tended to spend less time outdoors and wore more protective clothing which resulted in a reduced number of tick bites. Furthermore, long periods of droughts affected tick activity and therefore tick biting. Trends were generally comparable over the years with the exception of some particular events like the droughts of July 2018 and 2019 and the COVID-19 pandemic in 2020. A record number of 9,935 tick bites was reported in 2020 and for the first time since the start of TekenNet, more tick bites were reported in Wallonia compared to Flanders, with 49% and 48.5% notifications respectively. The percentage of tick bite reports made in Brussel was also higher than average (2.35%). The year of 2020 was different to other years in that people most often recorded tick bite occurrence in the forest and whilst out for leisure. The lockdown that was in place in Belgium between March and May 2020 and strict travel restrictions may account for these differences compared to the 2016-2019 and 2021 time periods. The restrictions likely led to more free time and leisure activities in nature, as well as more holidays spent in Belgium.

Conclusion
The citizen science platform TekenNet appears to be a valuable tool to collect tick bite prevalence data in Belgium. Even though the collection of the TekenNet data is based on voluntary participation of citizens, the observed trends over the years indicate that the data is nevertheless representative.
P037 - Tick-borne pathogens in children being evaluated for Lyme neuroborreliosis

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Introduction
Two of the most common and well known tick-borne infections in Sweden are Lyme borreliosis caused by Borrelia burgdorferi sensu lato species, and tick-borne encephalitis (TBE) caused by TBE virus. Although less common, other pathogens occur and can cause human disease (Anaplasma phagocytophilum, Rickettsia species, Neoehrlichia mikurensis and Babesia species) Studies show that a single tick can carry several pathogens at the same time, and potentially transmit them to humans. Reports of human disease caused by these pathogens are still rather uncommon. Missed diagnosis due to mild and unspecific symptoms, limited knowledge and awareness of clinical course, or limited access to specific diagnostic tools may certainly contribute to this. With this study, we strive towards better knowledge regarding the situation with potential tick-borne co-infections, or infections with, in Sweden less common pathogens.

Aims
Are co-infections with other tick-borne pathogens (A. phagocytophilum, Rickettsia spp., N. mikurensis, or Babesia spp.) present in children investigated for suspected Lyme neuroborreliosis (LNB) in Sweden? Could these pathogens explain symptoms in patients where LNB is ruled out? If so, will it affect the clinical outcome for these patients?

Methods
From children evaluated for LNB during 2011-2014 in Sweden, plasma and cerebrospinal fluid (CSF) samples were obtained and retrospectively analysed. Plasma samples were analysed for the presence of A. phagocytophilum, Rickettsia spp., N. mikurensis, and Babesia spp. DNA by using real-time PCR. In addition, CSF samples that were available were analysed regarding Rickettsia spp. Clinical data, symptoms, and laboratory findings regarding patients had prospectively been collected using a standardized questionnaire at inclusion, and at a 2-month clinical follow-up.

Results
Plasma samples (n=231) and CSF samples (n=67) were analysed from a total of 235 patients. 65 patients were classified as definite LNB, 33 patients as possible LNB, and the remaining 137 patients as non-LNB.
All samples were negative in the PCR analyses, except for plasma samples from two patients, one with and one without LNB, that were weakly positive for N. mikurensis. However, the positivity could not be confirmed by analysis with real-time PCR detecting another target gene.

Conclusions
Although ticks frequently carry multiple microorganisms with the potential to cause human disease when transmitted via a tick bite, we did not find any molecular evidence of such infections in Swedish children being evaluated for LNB. Therefore we conclude that these
infections do not seem to be frequently over-looked in this group of patients. However, given the indications of the emergence of ticks and of several of these pathogens, we suggest that they should still be considered as differential diagnoses in patients with for example unexplained fever during the tick season.
P038 - The prevalence of Borrelia miyamotoi in Ixodes ticks and humans in the Northern hemisphere: A systematic review and meta-analysis

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Introduction
Various studies have evaluated infection of Ixodes ticks and humans with the relapsing fever spirochete Borrelia miyamotoi. However, it has never been assessed systematically. This systematic review and meta-analysis examines B. miyamotoi prevalence in Ixodes ticks and humans, and the disease it can cause in the Northern hemisphere.

Materials and methods
PubMed and Web of Science were searched up to March ¹th, 2021. Publications assessing Ixodes tick infection using data from 2011 onwards were eligible, whereas no time limitation was placed on human reports. B. miyamotoi test positivity ratios were extracted. A random effects-model was used to calculate estimated proportions (ES) with 95% confidence interval (CI). This study was registered at PROSPERO (CRD42021268996) and followed PRISMA guidelines.

Results
Included articles reported on 165,637 questing ticks, 45,608 unique individuals, and 504 well-described human cases. In ticks, the highest prevalence was observed in Ixodes persulcatus (2·8%, 95%CI 2·4-3·1%) and the lowest in I. pacificus ticks (0·7, 95%CI 0·6-0·8%). The overall seroprevalence in humans was 4·4% (95%CI 2·8-6·3%), with a significantly (p<0·001) higher seroprevalence in the high risk group (4·6%, 95%CI 2·6-7·1%), participants with confirmed or suspected Lyme borreliosis (4·8%, 95%CI 1·8-8·8%), and individuals suspected of another tick-borne disease (11·9%, 95%CI 5·6-19·9%), as compared to healthy controls (1·3%, 95%CI 0·4-2·8%). Individuals suspected of another tick-borne disease were significantly more often B. miyamotoi-PCR positive than the high risk group (p=0·0246), with individuals in Asia more likely to be positive than those in the United States of America (OR 14·63, 95%CI 2·8-76·41).

Discussion
Our findings demonstrate Borrelia miyamotoi disease should be considered an emerging infectious disease, especially in North America and Asia. Prospective studies and increased awareness are required to obtain further insights into the burden of disease.
P039 - Better insight into the epidemiology of Crimean-Congo hemorrhagic fever virus in Corsica (France): a One Health approach.

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Among pathogens responsible for emerging infectious diseases, those with vector transmission are of major importance. Corsica, a Mediterranean island, is characterized by several factors supporting the spread of vector-borne diseases. Mass tourism and extensive agriculture favor interactions between humans, livestock, and wild fauna. Moreover, Corsica, located on the avian migration route, allows transport of new invasive tick species. Favored by global warming, ticks succeed in settling and circulating new diseases including Crimean Congo hemorrhagic fever (CCHF). CCHF is a common zoonotic viral infection transmitted by ticks. In 2016, human cases were detected in Spain. CCHF was also detected in ticks in Italy and other countries of the Mediterranean region like Spain, Algeria, Morocco, Tunisia and others. In Corsica, CCHF virus antibodies were detected in domestic animals with a seroprevalence of 13% in cattle and 2.5% in sheep. In 2016, a surveillance study on Corsican tick was set up however no CCHFV were detected despite the large circulation of its main vector, and till date no human cases were reported. The objective of this thesis is to strengthen the preparation for tick-borne diseases emergence, especially CCHF emergence. Virus circulation will be examined via a One Health approach and studied in all compartments involved in its epidemiological cycle. A molecular detection of CCHF virus in ticks collected from domestic animals will be conducted in parallel with an 18-month epidemiological survey targeting populations at high risk for CCHF infection due to their proximity to livestock and wild animals (Farmers, slaughterhouse workers, hunters, and veterinarians). To this end, a questionnaire will be conducted to assess exposure factors in these populations and serum samples will be collected to calculate the seroprevalence of anti-CCHFV antibodies and compare it to the general population. This transdisciplinary study is necessary to evaluate the source of a possible epidemic and thus monitor its possible emergence by putting in place preventive measurements and raising public health awareness in Corsica and neighboring regions.
Introduction.
Human babesiosis is a worldwide emerging tick-borne disease caused by intraerythrocytic protozoa. Most patients experience mild to moderate illness, but life-threatening complications can occur. Although cardiac complications are common, the full spectrum of cardiac disease and the frequency, risk factors, and outcome of patients experiencing cardiac complications are unclear. Accordingly, we carried out a chart review of cardiac complications among babesiosis patients admitted to Yale New Haven Hospital (YNHH) over the last decade to better characterize babesiosis cardiac complications.

Aims.
We sought to determine the frequency, risk factors, and outcome of patients experiencing babesiosis.

Methods.
We reviewed the medical records of all adult babesiosis patients admitted to YNHH from January 2011 to October 2021, confirmed by identification of Babesia parasites on thin blood smear and/or by polymerase chain reaction. The presence of Lyme disease and other tick-borne disease coinfections were recorded.

Results.
Of 163 enrolled subjects, 32 (19.6%) had at least one cardiac complication during hospitalization. The most common cardiac complications were atrial fibrillation (9.4%), heart failure (8.6%), QTc prolongation (8.0%), and cardiac ischemia (6.8%). Neither cardiovascular disease risk factors nor preexisting cardiac conditions were significantly associated with the development of cardiac complications. The cardiac complication group had a greater prevalence of high grade parasitemia (>10%) (p<0.001), longer median length of both hospital (p<0.001) and intensive care unit stay (p<0.001), and higher mortality (p=0.024) than the non-cardiac complication group.

Conclusions
Cardiac complications of acute babesiosis are common and occurred in approximately one fifth of this inpatient sample. Neither cardiovascular disease risk factors nor preexisting cardiac conditions were risk factors for cardiac complications. Further investigation is needed to elucidate the relationship between babesiosis severity and cardiac outcomes.
P041 - An integrated approach involving seroprevalence in farm animals and virus detection in collected ticks as an effective tool for tick-borne encephalitis virus surveillance in Slovakia

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Introduction
Tick-borne encephalitis virus (TBEV) is a causative agent of tick-borne encephalitis, a serious neurological disease with more than 10,000 cases per year in Eurasia. People can get infected by tick bite or after the consumption of unpasteurized raw milk products from infected animals resulting in alimentary outbreaks. In addition, ruminants develop an immune response with the production of persistent TBEV-specific antibodies and thus can serve as sentinel hosts for serological monitoring.

Methods and Results
In our study, a total of 1029 goat and sheep sera from 18 localities, collected in Slovakia during 2017-2019, were initially screened for TBEV-specific antibodies by ELISA and revealed 63 positive (6%) and 41 borderline sera (4%). Altogether 94 (89.5%) of them were confirmed as positive by plaque reduction neutralization test (PRNT) with TBEV. Sera showing inconsistent results in ELISA and PRNT were further analysed in West Nile virus-specific PRNT to evaluate the potential cross-reactivity. Moreover, tick collection was performed in areas determined by observed seroprevalence in tested animals or by reported human cases. Overall, 2,827 ticks (mostly Ixodes ricinus) from 9 collection sites were analysed by real-time RT-PCR resulting in a prevalence of 2.53% (ranging from 0.31 to 8.72% at different sites). Positive ticks were then used also for virus isolation and genetic characterization.

Conclusion
Although the seroprevalence of TBEV in farm animals does not provide sufficient information about the time and place of infection, it is an effective tool for the selection of areas suitable for more in-depth investigation of local ticks. Altogether, our combined approach provides valuable data on the putative risk of alimentary infections together with further characterization of the local TBEV strains.
P042 - Epidemiology of Lyme neuroborreliosis in Scotland: Data analysis from the Scottish Lyme disease and tick-borne infections Reference Laboratory

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Introduction
Lyme neuroborreliosis (LNB) is infection of the nervous system caused by tick-borne Borrelia burgdorferi. Clinical presentation and symptoms of LNB include cranial nerve palsies, meningitis, and encephalitis.

The Official Journal of the European Union outlines clinical and laboratory criteria for the diagnosis of LNB:
- Probable LNB is detection of Borrelia burgdorferi specific antibodies in the CSF with pleocytosis or raised CSF/serum antibody index (AI).
- Confirmed LNB is raised CSF/serum AI with CSF pleocytosis.

AI is the current gold standard for laboratory diagnosis of LNB but has remain positive for some time after treatment.

Aims
This study aims to review the incidence, epidemiology and clinical presentation of patients with Lyme neuroborreliosis in Scotland over 1 year period (September 2019 to September 2020).

Methods
Our laboratory provides testing for all patients with suspected Lyme disease in Scotland. Serum samples are screened by immunoassay for Borrelia burgdorferi antibodies. Positive or equivocal results are then confirmed with immunoblot. Patients with suspected LNB who were tested for Borrelia burgdorferi IgG in CSF were identified from our laboratory information and management systems (LIMS).

Results
63 patients were tested for LNB; 43 male (68%) and 20 female (32%). 15 patients were categorised as confirmed/probable LNB cases on testing. The incidence of LNB for probable/confirmed cases in Scotland is 0.30/100,000.

Tick bites were reported by 8 (57%) of patients. History of a tick bite was unknown or not recorded in a further 6 (43%) and 1 patient had no history of tick bite. Erythema migrans was described by 3 (21%) patients. 3 (21%) described a different rash and 2 (14%) did not report a rash. The presence of rash was either not recorded or unknown in the remaining 7 (50%) of patients.

The most common symptoms reported are radiculopathy (50%), weakness (50%) followed by cranial nerve palsies (43%) and paraesthesia (29%). Facial nerve palsy appeared to be the most common cranial nerve affected which is consistent with other reports. Non-specific symptoms such as headache, fatigue and arthralgia are also relatively common (21% for each symptom).

Conclusion
LNB appears to have low incidence but an important diagnosis from our limited study. Only 8 (57%) patients reported a tick bite and 6 (43%) patients reported a rash hence it is important to emphasise that lack of tick bite or rash do not exclude LNB.

Clinicians should keep LNB high in their differential diagnosis in endemic countries when assessing patients with neurological symptoms including cranial nerves palsies.

Early referral and discussion with infection specialists or reference laboratories allow early diagnosis with lumbar puncture and management of patients with LNB.
P043 - Zoonotic Babesia in ticks in northern Italy

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Introduction
Babesiosis, caused by intraerythrocytic protozoan parasites of the genus Babesia, is a globally important tick-transmitted disease. More than 100 Babesia species infect a wide spectrum of wild and domestic animals worldwide and six have been identified as human pathogens. In Europe human cases of babesiosis belong to B. divergens, B. venatorum and B. microti and they are all transmitted by Ixodes ricinus ticks. Babesia spp. may be transmitted by ticks with other pathogens (e.g. Borrelia burgdorferi; Anaplasma spp.; Ehrlichia spp.). This phenomenon has caused growing concerns for both humans and animals health, due to severe outcomes associated with multiple infections.

Aims
A regional scaled survey was performed to study the impact of tick transmitted zoonoses on pet animal and humans during 2018-2019.

Methods
Ticks were collected from wild and domestic animals and investigated for piroplasms by molecular diagnostics in Emilia-Romagna region, northern Italy. Ticks were identified using morphological dichotomic key (Manilla). DNA was extracted from crushed tick samples with a QIAamp DNA Blood Mini Kit (Qiagen). For PCR amplification, primer forward CRYPTO F (5’-AACCTGGTTGATCCTGCCAGT-3’) (Herwaldt et al. 2003) and primer reverse RLB-R2 (5’-CTAAGAATTTCCACCTCGCA-3’) (Centeno-Lima et al. 2003) were employed. The amplification yields a specific fragment of approximately 800 bp of 18S-rRNA.

Results
A total of 1229 ticks were collected from wild and domestic animals. I. ricinus represent the main tick species in our environment (46% of tick samples). Other vectors are identified as Rh. sanguineus (38%), I. hexagonus (13%), D. marginatus (1,5%) and H. marginatum (1%). Other tick species such as I. acuminatus and Rh. turanicus represent only the 0.5% of our samples. A total of 168 (16,85%) of ticks tested positive for Babesia/Theleria through Real Time PCR. Subsequently, positive samples were tested by traditional PCR based on 18S gene and sequenced with sanger method. Nine different Babesia species were found in ticks, of which two are zoonotic, B. venatorum and B. divergens.
We found B. capreoli, B. equi, B. canis vogeli, B. vulpes, B. badger, Th. ovis and unnamed Babesia called tavsan2 found previously in Turkey in hares and Rh. turanicus ticks B. divergens was found in one female I. ricinus collected from an asymptomatic person B. venatorum was found in 3 samples of I. ricinus ticks collected from red deer, roe deer and a person.
Conclusion
Babesia are usually host specific parasite, nevertheless, a few species are zoonotic, predominantly affecting immunocompromised patients. Our study demonstrated that a large variety of babesia species are circulating in Northern Italy and the risk of acquiring a Babesia infection is concrete in our environment. Further studies are needed to better understand the role of wild fauna in maintaining tick population and babesia parasite in nature.
P044 - Predicting tick-borne encephalitis risk using airborne pollen data in Western-Central Europe

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Introduction
Tick-borne encephalitis virus (TBEv) is a flavivirus affecting the human central nervous system which is endemic in many European countries, with an average of 2700 confirmed human cases reported each year in Europe and a mean annual notification rate of 0.6 cases per 100,000 population. The pathogen circulates enzootically in natural foci among ticks and hosts, especially rodents, whose population dynamics largely depends on availability of food, such as plant seeds. As airborne pollen abundance is a key proxy of seed production for some tree species, we investigated whether recorded human TBEv infections could be directly associated to pollen dynamics at different temporal lags in the Alpine biogeographical region, where the yellow-necked mouse (*Apodemus flavicollis*) is one of the most important amplification host species for the virus.

Methods
We focused our study on the province of Trento (northern Italy, ~6,000km², ~500,000 inhabitants), where TBEv cases have been recorded since 1992. Pollen concentration data were recorded approximately at the centroid of the region, from 1989 to 2020. Airborne pollen was sampled by a Hirst-type sampler and analyzed following conventional techniques and standardized protocols. Recorded taxa were hop-hornbeam (*Ostrya carpinifolia* Scop.), beech (*Fagus sylvatica* L.), spruce (*Picea abies* L.), pine (*Pinus sylvestris* L. and *P. nigra* J. F. Arnold), oak (*Quercus* sp.) and hazel (*Corylus avellana* L.). We statistically investigated the association between the yearly number of TBEv cases and the total yearly concentration of pollen of the taxa of interest collected during previous years. We firstly applied univariate linear models and subsequently built a full model by considering all significant covariates. We computed all possible sub-models and finally selected the best one according to the Akaike Information Criterion score.

Results
Between 1992 and 2020, a total of 206 TBEv human cases were recorded in the study area. We found a significant positive association between TBEv cases and pollen abundances with a two years lag for beech, oak and hop hornbeam. All other lags and taxa resulted in non-significant relationships. Finally, we identified the best model, which considers only hop-hornbeam and oak pollen quantities recorded two years before the occurrence of cases, both with positive coefficients, consistently with the univariate analysis.

Conclusions
To the best of our knowledge, this is the first attempt at quantifying the potential relationship between TBEv infections and pollen quantities. As pollen data is routinely collected at multiple sites worldwide and provides a quantitative measure, such analysis could be replicated at a larger scale in other biogeographical regions within the same biome, where *A. flavicollis* plays a major role as TBEv amplifying host species. If validated, pollen data might be therefore used to implement an early warning system evaluating the risk for TBEv transmission in Western-Central Europe.
P045 - Cost-benefit analysis of vaccinating a population against Lyme disease in high incidence areas of the United States

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Introduction
An estimated 476,000 cases of Lyme disease (LD) are diagnosed in the United States annually, resulting in significant disease and cost burdens. New LD vaccines and similar preventives are currently in development, and these products have the potential to substantially reduce disease incidence. However, the economic benefit of these candidates is unknown.

Methods
We conducted a cost-benefit analysis to estimate the net cost of vaccination against LD. Using a decision-analytic model, we compared a vaccination strategy to no vaccination among 100,000 individuals living in high incidence areas over a three-year time horizon. Model inputs included vaccine parameters (efficacy, uptake); clinical parameters (disease risk, outcomes); and costs (vaccine series, vaccine administration, disease costs). We derived base-case estimates and plausible ranges for model inputs from the literature and primary research. Model outputs included cases averted, net cost of the vaccination strategy, cost per case averted, and net cost per vaccinee. Deterministic sensitivity analyses were conducted to evaluate uncertainty and determine which inputs had potential to cause the greatest change in net cost per vaccinee.

Results
In the base-case scenario, at an incidence of 0.01, we estimated that 2,160 cases would be averted, and the net cost of the vaccination strategy would be $12.5M over a three-year period. These totals translate to a cost per case averted of $9,301 and a net cost per vaccinee of $156. In the optimistic scenario, with all parameters set to favor vaccination, we estimated that 22,334 cases would be averted at a net savings of $131.8M, translating to a cost per case averted of $616 and net savings per vaccinee of $1,402. In the pessimistic scenario, with all parameters set against vaccination, we estimated that 134 cases would be averted at a cost of $42.5M, translating to a cost per case averted of $316,848 and a net cost per vaccinee of $664. The primary output, net cost per vaccinee, was most sensitive to changes in disease incidence and vaccine price. In two-way sensitivity analysis, at the base-case incidence of 0.01, there was a potential for net savings up to a vaccine price of $45 (i.e., the breakeven price at which the net cost per vaccinee was $0). At the upper bound incidence of 0.08, there was potential for net savings up to a breakeven vaccine price of $476.

Conclusion
Many counties have annual LD incidence greater than our base-case estimate of 0.01, and while the price of a potential vaccine or similar preventive is currently unknown, it is possible the product could be cost saving, dependent upon final price and performance parameters. These results can inform recommendations for use of a LD vaccine once approved by the Food and Drug Administration in the United States.
P046 - Nationwide Surveillance for Tick Exposure, Made Easy

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Introduction
Tick bite is the single, essential event that defines risk for tickborne illnesses. A fuller understanding of the frequency and distribution of human tick bite could improve risk monitoring, identify drivers of exposure, and help target prevention efforts. We explore visits to “tick removal” websites maintained by the US Centers for Disease Control and Prevention (CDC) as a means of efficiently monitoring tick bite occurrence nationwide and generating insights into factors influencing tickborne disease risk.

Aims
- Describe the temporal/geographic distribution of tick removal website visits
- Compare website visits with regional tick phenology, emergency department consultations for tick bite, and tickborne disease incidence
- Use visits to identify biotic and abiotic factors influencing patterns of human tick exposure

Methods
CDC maintains two standing websites describing the proper removal of ticks. Daily visits to these sites were tallied for the three years January 2019–December 2021. Internet provider (IP) addresses were used to assign a US state as the geographic source for each inquiry. Data were compared with information from other sources (emergency department consultations, notifiable disease reports, meteorological data) after aggregating to match the spatial and temporal scale of these other sources. Multiple regression was used to assess associations with visit frequency and to derive parameters for a proof-of-concept, predictive model.

Results
A total of 1,930,010 visits to CDC’s tick removal web pages were registered Jan 2019–Dec 2021 from computers with an identifiable US state IP address. Per capita visits were ~5-fold higher in the humid Northeast compared with the arid southwestern United States (391 vs. 69 per 100,000 population). Seasonal patterns varied across regions consistent with the phenology of the principal human biting tick genera in each area (Ixodes, Dermacentor, or Amblyomma). Web visits correlated strongly with emergency department consultations for tick bite by week and region for all three years ($R^2>$0.89), and with the annual incidence of reported tickborne illness by state ($R^2=0.74$) for 2019, the only year for which surveillance data were available. Factors significantly associated with daily visits included the day of the year (surrogate for tick phenology), the day of the week (most visits on Sunday and Monday) and the maximum temperature on the preceding day during the cooler spring and fall months. Simple models based on these parameters were able to predict 2021 visits on a seasonal and regional level with reasonable accuracy ($R^2>0.84$) using observed temperature data.

Conclusion
While the concept of monitoring disease through internet activity is not new, our analysis suggests that visits to a consistent tick removal web site can be surprisingly informative. A web dashboard reporting daily activity by state is being developed, as are models to forecast short term changes in regional tick bite risk.

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Introduction
Lyme disease (LD) is a tick-borne disease caused by *Borrelia burgdorferi sensu lato* (BbsI). The epidemiology of LD has been described in North America and Europe, but less described in Asia. However, LD is an emerging zoonotic disease globally, and more studies are needed to determine presence of Bbsl and potential LD disease burden outside North America, Europe, and China.

Methods
Websites of public health agencies in Asia were searched for public health reports containing data on disease burden of LD. When only number of LD cases were available, population sizes from census data of each country were used as denominators to calculate IP. The 95% confidence intervals (CIs) of IPs were calculated using the binomial exact method. Statistical differences between proportions were performed using two proportion Z-test. All the data analyses were performed in R Core Team (2021).

Results
Epidemiological surveillance reports from 2011 to 2020 were obtained from Korea’s Center for Disease Control and Prevention and Japan’s National Institute of Infectious Diseases; LD is notifiable in both countries. The number of cases and incidence proportions (IPs) were analyzed at the national and regional level by age groups and sex.
In Korea (2011—2020), there were 160 LD cases; mean annual incidence was 0.032 cases/100,000 persons (95% CI: 0.019—0.051). In Japan (2011—2020) 151 cases were reported; mean annual incidence was 0.012 cases/100,000 persons (95% CI:0.007—0.019). The incidence of LD in Korea was higher than in Japan (p=0.003). Classification of the LD cases in Korea by age groups indicated that most (60%) of cases were 30-59 years old, but in Japan the same proportion (~60%) of cases occurred in people aged ≥50 years old. In Japan, the LB incidence was higher in men (0.016 cases/ 100,000 persons) compared to women (0.008 cases/100,000 persons; p = 0.17). In contrast, in Korea, most of the cases were among women (56.8%). The incidence was higher in men (0.016 cases/ 100,000 persons) compared to women (0.008 cases/100,000 persons; p= 0.39). In Japan, 11.9% of total cases were reported in the capital, but most of the cases were reported in Hokkaido (53.6%), the northernmost region of Japan. In Korea, the city with the most LD cases was the capital, Seoul (30.6%); the province with the most LD cases was Gyeonggi (18.7%), Korea’s most populous province.

Conclusion
The number of reported cases of LD is increasing in Korea and Japan. The differences observed between the number of cases by age and gender highlight the need for targeted control and prevention strategies for the disease in each country.
P048 - Epidemiology of Lyme borreliosis in France in primary care and hospital settings, 2010 to 2019

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**Introduction**
In France, Lyme borreliosis (LB) incidence is mainly derived from the national sentinel network (SN) and the national hospital discharge database (PMSI) to describe the epidemiology in primary care and hospital settings respectively. Data up to 2016 were analysed and published. Our study compared incidence rates of LB by sex, age and region in primary care and hospital settings between 2010 and 2019, using for the first time an additional source of data in primary care.

**Methods**
Incidence rates of LB in primary care were estimated between 2010 and 2019 using data from the SN and electronic medical records (EMR) of a network of general practitioners independent of the SN. Hospitalization rates were calculated between 2012 and 2019 from the national hospital discharge database (Programme de Médicalisation des Systèmes d’Information - PMSI). Hospitalized cases were identified using an algorithm combining 3 LB specific ICD10 codes (i.e. A69.2, M01.2 and L90.4) and compatible codes for disseminated forms. Average annual incidence rates were calculated for 2010-2012 and 2017-2019 to smooth annual variability.

**Results**
Incidence rates in primary care increased from 42.3 cases/100,000 population from 2010-2012 to 83.0/100,000 in 2017-2019 for the SN and from 42.7/100,000 to 74.6/100,000 for the EMR. Women were slightly predominant in primary care (1.09 women/1 man). From 2017-2019, the age distribution was bimodal with the first peak in children aged 0-4 years for the SN (65.3/100,000) and 5-9 years for the EMR (45.8/100,000) and a second peak in individuals 60-69 years of age (156.3/100,000 for the SN and 127.3/100,000 for the EMR). The regional incidence rate was highest in Limousin (330/100,000 for the SN and 355.5/100,000 for the EMR) and Alsace (244.3/100,000 for the SN and 162.3/100,000 for the EMR).

The hospitalization rate remained stable during the study period, fluctuating between 1.57 and 1.80 admission/100,000 population. Men were predominant (1.43 man/1 woman), mainly in adolescents aged 10-14 years (1.89 boy/1 girl) and in adults with a maximum in 80 years of age and older (2.58 men/1 woman). From 2017-2019, the age distribution of hospitalization was also bimodal with a peak in children aged 5-9 years (2.03/100,000) and adults aged 70-79 years (3.35/100,000). Regional incidence rates were the highest in Limousin (8.15/100,000) and Alsace (3.35/100,000).

**Conclusion**
Incidence rates of LB were high in specific age groups and regions which may inform public health prevention initiatives. Comparing for the first time 3 independent data sources highlighted disparities in incidence evolution, sex ratios and predominant age groups between cases seen in primary care and hospital settings.
Introduction
Lyme borreliosis (Lyme disease) is a bacterial systemic disease with the highest affinity for the skin, nervous system, joints and heart. The cause of the disease is the bacterium *Borrelia burgdoferi*, which is often found in the body of ticks and disease is transmitted to humans through a tick bite. Incubation ranges from a few days to a month. Symptoms are redness at the site of the bite, rash (erythema migrans) and general symptoms like fever, chills, fatigue and body aches. If the disease is not recognized in time and not adequately treated, the disease progresses and affects various organs, with various clinical symptoms. The most severe clinical presentation is neuroborreliosis. If the disease is not treated it can be fatal.

The disease is very common in America, but it is increasingly being registered in Europe, China, Japan, and the former Soviet Union. The vector of borreliosis is the tick *Ixodes ricinus*. Transmission occurs after infected tick has been attached to the skin for 36-48 hours, so a detailed examination of the body after the risk of contact with the tick is important.

Objections/Aims
To analyse data about Lyme borreliosis in the Federation of Bosnia and Herzegovina (FB&H) for the period 2016-2020

Methods
Official data on registered infectious diseases in FB&H which are mandatory for reporting according to the Law on Protection of the Population from Infectious Diseases were used for this research since Lyme borreliosis is on the list.

We used analytical-descriptive epidemiological method. Our study is retrospective for the period 2016-2020.

Results
Lyme borreliosis has been registered in FB&H with an average prevalence 0.8/100 000 inhabitants (0.3-1.1). The highest percentage of patients (35%) are in the age group 50-64 years, and the lowest percentage in age group 15-24 years, 6%. There is no significant sex difference, the ratio is 49% male: 51% female. This disease has been registered in seven of the ten cantons in the FB&H and the highest incidence was in the cantons located in the north and in the central part of the Federation. The highest incidence was recorded in the canton of Central Bosnia in 2018 and was 4.77/100 000, and the highest average incidence for five years (1.53/100 000) was in the Una-Sana Canton which is located along the border with Croatia.

Conclusions
From year to year, the number of people suffering from Lyme borreliosis in FB&H is growing and the disease is being registered in an increasing area.
P050 - MLST typing of Lyme borrelia in synovial fluids from Lyme arthritis patients living in Southern Sweden

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Introduction
Lyme borreliosis is endemic in Sweden and human isolates recovered from skin and cerebrospinal fluids published to date mainly belong to the two species Borrelia afzelii and Borrelia garinii. At the regional laboratory Borrelia DNA in patient samples is routinely identified by using a real-time 16S rDNA PCR and species is characterised by sequence analysis of ospA. When awareness of Lyme arthritis rose over the years Borrelia burgdorferi sensu stricto appeared to be the second most frequent species identified after B. afzelii in synovial fluids. For epidemiological interest next step was subtyping the B. burgdorferi s.s. isolates.

Aims
To set up the MLST typing method and perform ST typing on synovial fluids.
To identify B. burgdorferi s.s. MLST types found in synovial fluids from patients in Sweden and compare the findings with other geographic locations.

Methods
A set of 14 cultured samples were analysed in order to verify the sequencing protocol. The verified protocol was then used to analyse a collection of 69 synovial fluid samples collected from 2013 until 2021 from patients living in Sweden. Each sample had earlier been assigned to one of the species B. afzelii, B. burgdorferi s.s., B. garinii, B. bavariensis and B. spielmanii. Multilocus sequence typing (MLST) based on the sequence analysis of eight genes (clpA, clpX, pepX, pyrG, nifS, recG, rplB and uvrA) was performed according to Margos et al. 2008 Proc Natl Acad Sci USA 105:8730-5. Sequence types (ST) were determined by use of the PubMLST borrelia spp database typing tool (https://pubmlst.org/organisms/borrelia-spp). Prior to MLST, all samples had been verified and assigned to a Borrelia species by Borrelia 16S rDNA PCR and ospA sequencing.

Results
Of the 69 synovial fluids samples 23 were successfully sequenced and assigned and given an ST. Of the 12 synovial fluids samples identified as B. burgdorferi s.s. 5 belong to ST-type 20, 1 belong to ST-type 21, 5 belong to ST-type 24 and 1 belong to ST-type 284. One sample has a dropout in one loci, 2 samples have a mismatch in one loci and one sample in 2 loci.

Conclusion
MLST typing method of Borrelia species in synovial fluids was successful in one third of the samples. The four STs found in the 12 B. burgdorferi s.s. isolates identified in patients living in Sweden have earlier been found in ticks in Northern Europe. ST 21 has been found in humans in Germany. Further sequence analysis and comparisons by phylogenetic analysis should be performed.
**P051 - Ticks collected from humans via a citizen-science initiative in Belgium: year 2021**

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**Introduction**

TekenNet or TiquesNet is a Belgian citizen-science initiative with the objective of mapping the human risk for a tick bite in the country. Through a website, people can notify a tick bite and submit information on the geographical location and circumstances of the bite.

In 2017, between April 1st and October 31st, the tool was additionally used to collect ticks removed from humans, to study the prevalence of several tick-borne pathogens (TBP).

Of the total 1,500 ticks analysed, 99% were *Ixodes ricinus* with 81% at the nymph stage. Some *Ixodes hexagonus* (0.7%) and *Dermacentor reticulatus* (0.3%) were also found. Overall, 14% were infected with *Borrelia burgdorferi* sensu lato. The prevalence of other TBPs ranged from 1.5% (*Babesia* spp.) to 6.8% (*Rickettsia helvetica*). Tick borne encephalitis virus (TBEV) was not detected. A second human tick collection was organized in 2021.

**Aims**

The purpose of this study was to evaluate trends in this citizen-science initiative. Readouts of this comparison are tick species distribution in Belgium and the prevalence of several TBPs.

**Methods**

Between April 1st and October 31st 2021, citizens were invited to send ticks removed from their skin by postal mail and fill in a short online questionnaire. At arrival, envelopes were stored at -80°C, until analysis. Ticks were microscopically identified for species and developmental stage, washed and processed for nucleic acids extraction. The extracts will be used to perform different qPCRs targeting *Anaplasma phagocytophilum, Borrelia* s.l., *Babesia* spp, *Spiroplasma, Rickettsia* spp., *Ca. Neoehrlichia mikurensis*, and TBEV.

**Results**

In 2021, a total of, 1,197 ticks were collected. As in the previous study, the species most frequently identified was *I. ricinus* (94.1%), 81.7% of which at the nymph stage. Few *I. hexagonus* (0.7%) and *D. reticulatus* (0.3%) were also present. Some ticks could not be morphologically identified due to extensive damage (4.3%). Interestingly, unlike 2017, we received seven soft ticks, identified as *Argas reflexus* (0.6%) all from the same participant in the Brussels region. Prevalence on TBPs in the 2021 tick collection is ongoing, and final comparative results will be available by September 2022.

**Conclusion**

As in 2017, the citizen-science tool TekenNet allowed to collect a sample of more than 1,000 ticks spread over the country. Tick species distribution remained the same between the two collections, confirming *I. ricinus* the most important tick species for the public health risk in Belgium. Interestingly, soft-ticks were also identified, but the public health risk of the latter remains poorly described.
INTRODUCTION

*Borrelia miyamotoi* is a spirochete transmitted to vertebrate hosts by the hard ticks, *Ixodes ricinus*, the main vector of *B. burgdorferi*, the agent of Lyme disease in Europe (1). *B. miyamotoi* belongs to the relapsing fever clade and it was first described in Japan in 1994 in *I. persulcatus* ticks (2), and in 2011 in Russia in humans (3) with influenza-like symptoms including high fever, fatigue, headache, chills, myalgia, arthralgia and nausea (1). Considering that in Italy the knowledge on the distribution, ecology and epidemiology of *B. miyamotoi* is limited (4), the aim of this study is to investigate the presence of *B. miyamotoi* in *I. ricinus* collected from humans in Lombardy, Northern Italy.

METHODS

The ticks were collected in 2020-2021 recovered from patients from two location in Northern Italy (Sondrio and Pavia), as a part of collaboration in a one-health perspective with the local health institutions.

The ticks were morphologically identified at species level and life stage, using taxonomic keys. The DNA was extracted using NucleoSpin® Tissue kit (Macherey-Nagel) and tested for different pathogens (*Borrelia spp.*, *Rickettsia spp.*, *Francisella tularensis*, *Coxiella burnetii* and Tick borne encephalitis). In particular, *Borrelia spp* was detected by Real-Time PCR (5) and the positive samples were then tested for *B. miyamotoi* by PCR Real-Time targeting the *glpQ* gene (6). Amplification (~900bp) (7) and sequencing of the *glpQ* gene were performed for the phylogenetic analyses.

RESULTS

A total of 650 ticks were morphologically identified as nymph (76,6%), adult female (16,92%), larvae (4,61%) and adult male (0,76%). The ticks were taxonomically assigned to 4 species: 641 to *Ixodes ricinus* (98,61%), 4 to the genus *Dermacentor* (0,61%), 1 to *Rhipicephalus* (0,15%) and 4 to the species *Ixodes hexagonus* (0,61%). Six samples (0,92%), all belonging to the species *I. ricinus*, collected from the province of Sondrio, resulted positive for *B. miyamotoi*, and the sequences obtained showed a percentage of identity between 95 and 99% with *B. miyamotoi*. The phylogenetic analyses revealed that the sequences obtained cluster with European clade.

CONCLUSIONS

Despite the low rate of *B. miyamotoi* positive samples, the detection of this pathogen is important as it is spreading to the area. It is also of interest for physicians when considering diagnosis of potential tick-borne disease. Indeed, *B. miyamotoi* infection can lead to relapsing fever or meningoencephalitis, but is not yet well-known among physicians. Monitoring studies are needed to define the distribution and the prevalence of this pathogen. Furthermore, a
collaboration between veterinarians and physicians is important to collect more clinical data for a correct diagnosis and follow the patients in the evolution of the disease.
The largest outbreak of Francisella tularensis in Slovenia

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Introduction

Tularemia, also known as “rabbit fever,” is a zoonotic disease caused by gram-negative bacterium Francisella tularensis, one of the most infectious pathogens known in human medicine. The bacteria is a highly infectious, facultative intracellular pathogen that can also be used for bioterrorism. The main reservoir of F. tularensis are considered to be lagomorphs and rodents. In Slovenia, tularemia is rare and sporadic disease. In the last thirty years (from 1990 to 2020) only 42 cases were reported with up to 2 cases per year. Most of the patients have ulceroglandular form of tularemia, which correlates with epidemiological data indicating transmission by a tick bite in 50 % of patients. However, in 2021, Slovenia experienced its largest tularemia outbreak to date, with 56 clinical cases reported.

Methods

Initially, the clinical suspicion on tularemia was raised on the basis of epidemiological data related to contact with wild hares. After the first cluster was discovered, the National Institute of Public Health began an active search for cases. Clinical and epidemiological suspicion was further on confirmed with laboratory diagnosis, which included detection of specific antibodies by indirect immunofluorescence assay or molecular detection. In addition, the laboratory initiated field investigation to discover the source of infection and recovered bacterial isolates from several sources.

Results

The first case was diagnosed already in February, but the peak of the epidemic was in July. Patients were from all age groups, but most of them were men in the aged 61-70 years. The most common clinical presentations were pneumonic (18), typhoidal (17), and oropharyngeal (10), followed by ulceroglandular (4), glandular (6) and oculoglandular (1) tularemia. One patient had severe form of the disease and presented with myocarditis. In total, twenty-six patients required hospitalization. The diagnosis was primarily established by serology: 55 patients already had antibodies in initial sample. In one case, the diagnosis was made on positive PCR test of ulcer swab, one PCR test of lymph punctate was also positive. Epidemiological investigation revealed that the most important risk factors were contaminated water, contact with hares and tick bite. The microbiological investigation confirmed bacteria in private water wells, hares, rodents and ticks collected in the affected areas.

Conclusion

This was the first tularemia outbreak in Slovenia involving several municipalities, with five family cluster, where the source of infection was contaminated water supply. Microbiological investigation showed that the most likely explanation for diverse clinical presentation is different source of infection. The typhoid, oropharyngeal, oculoglandular and pneumonic tularemia were
probably associated with contaminated water, ulceroglandular and glandular forms with tick bite and handling of infected animals.
P054 - Serological detection of Borrelia spp. in dogs and horses from Germany (2016-2021)

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Introduction
Lyme borreliosis is a tick-borne disease intensively studied in humans, less though in dogs and horses.

Aims
Aim of the study was to assess the percentage of positive tests for Borrelia spp. in dogs and horses.

Methods
Results of Enzyme-linked immunosorbent assay (ELISA) and Immunofluorescence antibody (IFA) testing for Immunoglobulin M (IgM) / Immunoglobulin G (IgG)-antibodies as well as results of an IgG-Western blot interpreted according to the manufacturer’s guidelines, requested by German veterinarians between 2016 and 2021 out of serum samples from a commercial laboratory, were included in the retrospective study. The study period was divided into three timeframes: I: 2016-2017; II: 2018-2019; III 2020-2021.

Results
1,291/4,436 horses (29.1%) and 6,105/30,018 dogs (20.3%) tested positive for IgG/IgM-antibodies (horses I: 23.2%; II: 27.6%; III: 35.5%; dogs I: 20.3%; II: 19.8%; III: 20.9%). Highest percentage of seropositive horses/dogs was seen in the southern federal states Rhineland Palatinate (33.3%/22.1%), Baden-Wuerttemberg (30.6%/24.2%) and Saarland (30.3%/21.0%) as well as Lower Saxony/Bremen (31.1%/21.8%) and Thuringia (22.4% in dogs). Thus, geographical distribution, percentage of seropositivity and development over time was similar for both species.

Western blot analysis was available in 7,093 dogs and 2,085 horses respectively with 37.6%/54.0% positive results. Antigen pattern resembling infection was seen in 20.4% (dogs)/37.6% (horses) while 2.1% (dogs)/16.3% (horses) resembled antigen contact only. For dogs 13.2% of the blot results
were indicative for vaccination, 1.5% for vaccination and infection. Higher percentage of dogs were classified as infected in contrast to a higher percentage of horses categorized as antigen contact without infection.

**Conclusion**

Monitoring is important to identify potential risk areas for all species including humans.
**P055 - Molecular and serological detection of Anaplasma phagocytophilum in dogs and horses from Germany (2008-2020)**

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**Introduction**

*Anaplasma* (A.) *phagocytophilum* is an obligate intracellular bacterium causing granulocytic anaplasmosis. In Germany, mainly *Ixodes ricinus* is considered as the transmitting vector.

**Aims**

Aim of the study was to assess the percentage of positive test results for *A. phagocytophilum* in dogs and horses.

**Methods**

Results of direct (polymerase chain reaction [PCR]) and indirect (immunofluorescence antibody test [IFAT] in both species; enzyme-linked immunosorbent assay [ELISA] in dogs) detection methods were included in the retrospective study. Tests requested by German veterinarians for dogs and horses between 2008 and 2020 were performed by a commercial laboratory.

**Results**

In total, 1,332/27,368 dogs (4.9%) tested positive by PCR and 24,720/90,376 (27.4%) by IFAT/ELISA. One-hundred-forty-nine out of 1,001 horses (14.9%) tested positive by PCR and 958/3,488 (27.5%) by IFAT. Seasonality had a statistically significant impact on PCR results in both species (P < 0.001 each) with highest percentage of positive tested dogs (9.0%) and horses (24.7%) in summer and especially in June (dogs: 11.9%, horses: 31.3%). Odds-ratio was 1.66 in dogs and 1.2 in horses in summer compared to spring, autumn and winter. For seropositivity statistically significant impact of seasonality was not seen in horses (p = 0.214), but in dogs (P < 0.001).

**Conclusion**

Dynamic of infections with *A. phagocytophilum* in dogs and horses matched with peaks in vector activity in Germany. Horses showed higher percentage of positive PCR-results compared to dogs, maybe due to lack of ectoparasite prophylaxis. Percentage of seropositivity was similar in both species indicating similar exposure to *A. phagocytophilum* over time.
P056 - Seroprevalence for *Borrelia burgdorferi* sl antibodies and associated risk factors among forestry workers, France.

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**Introduction**

Lyme borreliosis (LB) caused by the bacteria *Borrelia burgdorferi* sensu lato (*Bb*-sl) that is transmitted to humans by infected *Ixodes* ticks is the most common tick-borne disease in France and Europe. Hospital and primary care surveillance data suggest that most LB cases occur in children and elderly persons. Forestry workers are also at high risk of LB infection because of frequent occupational exposure to ticks. Population seroprevalence surveys, measuring the presence of specific antibodies are useful tools to assess the overall population exposure to infectious pathogens within a country or specific risk groups.

**Aims**

The aim of this survey was to estimate the seroprevalence of *Bb*-sl antibodies in forestry workers in the northern half of France. We also compared seroprevalence by region, assessed factors associated with the seroprevalence and evaluated knowledge and practices against tick bites and LB.

**Methods**

The study population was adults forestry workers covered by the occupational health services of the agriculture social insurance scheme. All participants were administered a face-to-face questionnaire inquiring about professional activities during the past 12 months, exposure to tick bites, clinical history of LB, travel history and the use of preventive measures against tick bites. Blood samples were obtained for serological analyses. Seropositivity were defined as a positive Elisa result and positive or equivocal Western Blot. We calculated weighted seroprevalence estimates for the whole population and stratified for sociodemographic or tick-bite exposure factors. Adjusted prevalence ratios (PR) were estimated using weighted poisson regression to determine the association between potential risk factors and seropositive status.

**Results**

A total of 1,778 forestry workers participated (response rate: 41%). History of tick bite was reported by 91% of and 69% had been bitten in the last 12 months. Most workers reported the use of protective clothing (62%), perform body checks after forest exposure (74%) and rapid removal of the tick (98%). The overall *Bb*_ sl seroprevalence was 16% (CI95% 15-18). It was 3.4 times greater in Eastern compared to Western regions. A higher seroprevalence was observed in professionals who had worked in forests for more than 20 years compared to those who had worked for less than five. It was 1.7 times higher among those working in the forests more than 20 hours per week. Forestry workers who declared not removing a tick rapidly after being bitten and who had a history of a tick bite had a higher seroprevalence compare to those not bitten (PR=2.8).

**Conclusion**
This study confirms that forestry workers are a population at risk for LB infection and assess for the first time the seroprevalence in the northern half of France. The results of this study will be used, together with data on LB incidence and knowledge, attitudes and practices studies, to target prevention programs.
P057 - Lyme disease seropositivity across China: A systematic review and meta-analysis

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Introduction
Following the initial identification in China of Lyme disease in 1986, cases have been diagnosed clinically in 29 Chinese provinces. However, it is difficult for national and local governments to effectively develop and implement prevention strategies without an understanding of disease burden. Currently, no national Lyme disease surveillance exists in China, and there are no published estimates of national disease incidence. Thus, the only available approach to quantify disease risk is human seropositivity data.

Aims
This systematic literature review in Chinese and English language journals between 2005–2020 summarizes human Lyme disease seropositivity data across China.

Methods
A global systematic literature review was conducted across 5 databases (global and local to China) following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. For articles that met the inclusion criteria, the number of individuals testing positive for IgM or IgG antibodies, through either an enzyme-linked assay (EIA) or in combination with confirmatory Western immunoblot, was extracted. Two-tier diagnostic testing strategies are not commonly employed in China and tests reporting IgM antibodies may have a higher rate of false positivity; hence, the primary analysis consisted of estimates based on a single tier EIA measuring IgG. Exposed populations were defined prospectively, characterized to reflect potential regional variation in tick exposure and Borrelia burgdorferi sensu lato transmission. Risk was classified as either high, medium, or low based on the likelihood of exposure to a natural foci of Lyme disease, either by residence or by occupation. Summary estimates were calculated for each province where data were collected. A fixed effects meta-analysis model was employed to calculate the least squares mean summary estimate from the available seropositivity estimates.

Results
The literature review identified 3,657 articles that focused on China; 48 articles met the selection criteria, of which 42 articles met the criteria for the primary analysis. In total, these 42 articles provided 72 estimates of seropositivity that were extracted for analysis. Based on 72 estimates that measured IgG antibodies using an EIA diagnostic test alone, the seropositivity point prevalence was 9.1% (95% CI: 7.5–10.7). A more conservative two-tier testing approach of
EIA plus a confirmatory Western immunoblot (n=16 estimates) yielded 1.8% (95% CI: 0.9–2.7) seropositivity. High- and low-risk exposure populations had EIA seropositivity of 10.0% and 4.5%, respectively. Provinces in Northeastern and Western China had higher seropositivity.

**Conclusion**

This analysis confirmed substantial Lyme disease burden, measured by human seropositivity, among populations at risk and in many Chinese provinces. Clinical incidence studies are needed, focusing on provinces with documented high seropositivity, to guide prevention measures.
**P058 - Characterization of Borrelia spp. in ticks in two Slovenian regions**

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**Introduction**

*Ixodes ricinus* is the most common tick species in Central Europe including Slovenia. It is also a primary vector of the causative agents of Lyme borreliosis (LB) and tick-borne encephalitis (TBE). In Europe, among twenty-one reported *Borrelia* species only several cause LB in humans (*B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto (s.s.), *B. bavariensis*, *B. spielmanii*). In Slovenia, these species were isolated from patients, some only from ticks, or from both. "Emerging pathogen" *B. miyamotoi* was first reported from Russia, and was detected in a mouse from Slovenia. The aim of the study was to assess *Borrelia* species in ticks from two southwestern Slovenian regions, the Littoral-Inner Carniola and Coastal-Karst.

**Methods**

Ticks were collected in May 2019; after decontamination they were inoculated in the MKP medium. Spirochetes from positive cultures were genotyped with *Mlu*I-LRFP. Ticks from negative or contaminated cultures were subjected for DNA extraction, and tested by real-time PCR for the presence of Lyme *Borrelia* and/or *B. miyamotoi* DNA. In PCR positive samples, a fragment of *flab* or *glpQ* was amplified and further sequenced to identify *Borrelia* species.

**Results**

All ticks (555 ticks *I. ricinus* and 4 ticks *Hyalomma punctata*) were cultivated in MKP medium; culture positive were 28 ticks (5.0 %). *Mlu*I-LRFP typing revealed 17 (53.3 %) *B. garinii* isolates with proceeding subtypes: Mlg2 12 isolates, Mlg6 2, and Mlg4, Mlg8 and Mlg9 1 isolate, respectively; Mlg8 and Mlg9 were new defined subtypes. Six isolates were determined as *B. afzelii* subtype Mla1, four as *B. valaisiana* (subtypes Mlv1 and Mlv2, each two isolates) and one as *B. burgdorferi* s.s. subtype Mlb2.

Of 531 ticks from negative or contaminated cultures, 156 were PCR positive, while 15 samples were inhibited. Sequencing *flaB* gene enabled determination of *Borrelia* species in 64 ticks: *B.*
*B. garinii* was found in 32 ticks, *B. afzelii* in 22 ticks, *B. valaisiana* in nine ticks and *B. lusitaniae* in one tick. The rest of ticks were *flaB* gene PCR negative.

Eight ticks (1.4 %) were PCR positive for *B. miyamotoi* but only in 4 samples *glpQ* gene was successfully sequenced and *B. miyamotoi* was confirmed. One of these ticks was coinfected simultaneously with *B. garinii* and *B. miyamotoi*.

**Conclusion**

Our results confirm that molecular methods are more sensitive in detecting *Borrelia* in ticks than culture. The dominant *Borrelia* species is *B. garinii*; moreover, two new subtypes within this species were detected. *B. miyamotoi* in ticks was detected in low frequency as reported before. In one tick, double infection with *B. garinii* and *B. miyamotoi* was detected.
Out of at least 21 described species, classified into *Borrelia burgdorferi* sensu lato complex, only five are confirmed as causative agents of Lyme borreliosis (LB) in Europe (*Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii*, *Borrelia spielmani*, and *Borrelia bavariensis*) while in North America LB is predominantly caused by *B. burgdorferi* s.s. with only a few cases caused with *Borrelia mayonii*. Additional three species (*Borrelia lusitanae*, *Borrelia valaisiana*, and *Borrelia bisetii*) relate to several cases of LB in Europe but their pathogenic potential is still unclear.

Previous studies in Serbia indicated high diversity of *B. burgdorferi* s.l. species and unexpected dominance and diversity of *B. lusitanae* in ticks. The available data on the epidemiology of LB in Serbia are scarce and the information on *Borrelia* species that cause LB is lacking. Considering the diversity and species composition of *Borrelia* species circulating in Serbia, we were interested to reveal the species causing LB.

The study group consisted of persons that sought medical attention after the tick bite. Blood sampling followed by serum isolation was performed four weeks after tick detachment. Human serum samples were checked for the presence of *B. burgdorferi* s.l. antibodies using an ELISA and Western blot, and samples positive for either IgM, IgG, or both were processed further. DNA was extracted from individual serum samples and *rrf-rrl rDNA* intergenic spacer nested PCR was applied for the detection of borrelial DNA. The purification and bidirectional sequencing (Sanger) of obtained PCR products were performed, and sequences were compared with previously published nucleotide sequences available in the GenBank® database using the BLAST tool.

A total of 46 serum samples (12 IgM positive, 21 IgG positive, and 12 IgM and IgG positive) were analyzed for the presence of DNA of *B. burgdorferi* s.l. According to clinical symptoms, five patients were diagnosed with and two were suspected of LB. DNA of *B. burgdorferi* s.l. was detected in sera of five patients, none of them were diagnosed with or suspected of LB. Borrelial DNA was detected in two sera positive for IgM and three sera positive for IgG. Sequencing was successful for all five samples, according to BLAST analysis revealed that four samples align with *B. afzelii* and one with *B. lusitanae*.

This is the first finding of *B. lusitanae* DNA in the human serum. Until now, only one case of LB was associated with *B. lusitanae*, and spirochaetae were isolated from a localized skin lesion. The occurrence of *B. lusitanae* in human serum indicates a certain level of resistance to complement
and potential for dissemination. Further research is needed to elucidate the destiny of *B. lusitaniae* strains transmitted by tick bite to humans.
The LymeProspect KIDS Prospective Study into Long-term Effects of Lyme Borreliosis in Children in the Netherlands

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**Introduction**

Children treated for Lyme borreliosis (LB) frequently report disabling symptoms. Although these symptoms often persist over time, pediatric studies on long-term effects of LB are scarce. We evaluated children treated for LB prospectively for prevalence and severity of and potential determinants for development of persistent symptoms.

**Methods**

In a prospective, observational cohort study, children (0-17 years of age) with confirmed erythema migrans (EM) or disseminated LB manifestations were enrolled from 2017-2019 at the initiation of their antibiotic treatment, and followed for one year. Prevalence and severity of persistent symptoms (lasting ≥6 months and reported within 6 months after treatment) were assessed by validated three-monthly online questionnaires. The focus of these questionnaires were fatigue (PedsQL-fatigue, subscale general fatigue), pain (VAS-pain, subscale 0-10 pain) and neurocognitive (dys)function (PedsQL-fatigue, subscale cognitive fatigue). To control for background symptom prevalence in children without evidence of LB, results are compared to a control cohort including children reporting a tick bite not followed by LB. In a subset of patients, blood was collected at enrollment and after 6 weeks follow up. Determinants of persistent symptoms were assessed by serology for *Borrelia* among others. Here we report preliminary clinical and serological characteristics of the included LB patient cohort.

**Results**

Ninety-seven children (median age 8 years (range 1-17); 47% female) with a diagnosis of LB were enrolled of which 76 were diagnosed with EM (78%) and 21 with a disseminated LB manifestation (22%). Median EM diameter was 6.5 cm (range 3-22) and median EM duration at inclusion was four days (range 0-103). Blood samples at inclusion and after 6 weeks were collected for a subset of 47 children. At baseline 32 (68%) of these 47 tested positive in *Borrelia* C6 ELISA (IgM/IgG) and 24 (51%) showed IgM/IgG immunoblot reactivity. Four (8.5%) children tested IgM/IgG positive by immunoblot at baseline and seroreverted to IgM/IgG negative in the first six weeks. The C6 Lyme index was higher in children with disseminated LB (9.0 ± 3.0, mean ± SD) compared to EM (2.9 ±3.1, mean ± SD) (p<0.001).

**Conclusion**
The LymeProspect KIDS study is characterized by a prospective approach and a control cohort allowing to control for background prevalence of persistent symptoms. Most included children tested positive in Borrelia C6 ELISA (IgM/IgG) and an association between C6 Lyme index and LB manifestation was found. Little dynamics in immune responses was seen, possibly due to antibiotic treatment. The results of this study are expected to provide insights into the long-term effects of LB and prevalence and severity of persistent symptoms in children.
**P061 - Stabilization in the incidence and burden of Lyme borreliosis in the Netherlands between 2017 and 2021?**

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**Introduction**
After a threefold increase in general practitioner (GP) consultations for tick bites and erythema migrans (EM) diagnoses from 1994 to 2009 in the Netherlands, a decrease in tick bite consultations and a stabilization in EM diagnosis was observed for the first time between 2009 and 2014. In 2017, however, an increase in tick bite consultations as well as EM diagnoses was observed again. To assess how this trend continued after 2017, we repeated the GP survey in 2021, using identical methods to the previous surveys.

**Methods**
To all (11,792) GPs in the Netherlands, a postal questionnaire was sent inquiring about the number of consultations for tick bites, EM diagnoses, clinical manifestations of disseminated Lyme borreliosis (LB), persisting symptoms attributed to LB (by the patient and/or the GP), and the size of the GP’s practice population. These questions were assigned values based on the best fit of an assumed underlying negative binomial distribution. As every person in the Netherlands is registered with only one GP, we used the practice populations of reporting GPs to calculate incidence rates and national estimates of total numbers among the population of the Netherlands. Bootstrap analysis was used to calculate 95% confidence intervals for the incidence rates. Additional questionnaires will be sent to be able to categorize reported disseminated cases and cases with persisting symptoms attributed to LB according to likelihood of the diagnosis.

**Results**
Preliminary analyses of the 2021 survey resulted in an estimated incidence of 474 tick bite consultations (95%CI: 450-499) and 154 EM diagnoses (95%CI: 147-162) per 100,000 inhabitants, compared to 531 tick bite consultations (95%CI: 515-548) and 149 EM diagnoses (95%CI: 144-154) per 100,000 inhabitants in 2017. This translates to 75,500 tick bite consultations and 25,600 EM diagnoses nationwide in 2021, and 91,000 tick bite consultations and 25,500 EM diagnoses in 2017. The survey had 2,015 GP respondents with a patient population of 5.6 million people, which accounted for a population coverage of 32%, which was lower compared to previous years.

**Conclusion**
In 2021, the incidence of erythema migrans and thus LB in the Netherlands remained stable and the incidence of tick bite consultations decreased compared to 2017. Additional analyses will be performed to explore possible bias through selective non-response by GPs in low and/or high risk areas.
P062 Prevalence of Borrelia antibodies among individuals with high occupational exposure to ticks in the Netherlands

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Introduction
Success of the antimicrobial therapy for Lyme borreliosis is higher in the early phases of disease, therefore timely diagnosis is crucial. Tick bites often remain unnoticed and erythema migrans, an early symptom of borreliosis, is often either unrecognized by the patient or absent, leading to delay in treatment.

Aim
To facilitate prompt diagnosis, our laboratory performs yearly serological screening for borreliosis infection for individuals with high occupational exposure to ticks. Here we report prevalence of antibodies in this group in 2021.

Methods
In total, 3759 individuals from all 12 Dutch provinces employed by 49 municipalities, 8 water authorities and 32 other organizations participated in the screening. Serum samples were first analyzed by ELISA for the presence of IgG and IgM antibodies (EUROIMMUNE and ZEUS tests). Positivity was then confirmed in IgG or/and IgM immunoblot (Viramed). The obtained results were compared to those of previous sampling. Increased ELISA levels confirmed by additional/other antigen bands on the immunoblots were considered as criterion for new infection.

Results
For 187 (5%) participants, indication for new infection in 2021 was established. These individuals were advised to seek medical attention. In samples from 467 (12%) individuals, antibodies were detected, but the levels were similar to past measurements, indicating that they represent serological traces of previous infections. In other participants, 3105 (83%) no antibodies were established. Since the start of the Innatoss annual borreliosis screening, serological indication of new infection was found in 2016 in 6% of participants, in 2017-2020 in 2% and in 2021 in 5% of participants.

Conclusion
These unique data provide insight into nation-wide distribution of Borrelia antibodies in a large group of individuals with high exposure to ticks. Antibody profiles suggest that in 2021 prevalence of new Borrelia infections was higher than in the previous years in this group. Possible reasons for the difference will be discussed.
Introduction
Borrelia species are zoonotic bacteria which cause, among other things, relapsing fever and Lyme disease. *Borrelia persica* is endemic in Israel and causes tick-borne relapsing fever, also known as “cave fever”. Our report covers the new actions taken by the Vector Control team at the Ministry of Environmental Protection (MOE). Specifically, performing environmental investigations and formulating specific pest management plans for unexpectedly infested areas.

Methods
Environmental investigations were conducted based on epidemiological investigations or reports by infected individuals. Pitfall traps were placed in suspected areas and provided with CO2 continuously for approximately four hours. A sample of ticks collected from traps was submitted to the Public Health Laboratories at the Ministry of Health (MOH). Warning signs were placed at locations with infested caves to prevent access, while at three sites pest management was required due to proximity to human habitations.

For pest management, we advised the following protocol: Release of CO2 to lure ticks to the surface before applying pyrethroid-based insecticides. Follow up tick monitoring was conducted approximately 2 weeks after.

Results
During October 2020 to December 2021, six environmental investigations were conducted, five of which were conducted after an epidemiological report was received from the MOH district. One investigation was conducted after a citizen discovered a tick in her bed and informed the municipal authority.

An environmental investigation involved setting traps at several suspected sites, at least one trap containing soft ticks was found in every investigation.

As expected, we found that the longer the tick traps were left in place, the higher the number of ticks collected.

Three investigations determined that caves were the source of infestation. The other three revealed unusual habitats, two of which were found under man-made structures, and one was a small lair beneath a rock, close to adolescence hangout in the peripheral outlines of a village. Follow-up tick monitoring revealed empty traps in these habitats, indicating effective pest management.

Conclusions
In the past, the MOE relied only on epidemiological findings and prohibited entry into suspected caves. As of 2020, the MOE Pest Control team and the MOH Public Health Laboratories have developed a collaborative method for conducting environmental and laboratory investigations of endemic relapsing fever. Environmental investigations are used to locate the source of infestation, afterwards preventative measures are taken.

In this report, we demonstrate the value of a “One Health” approach since vector habitat influences the occurrence of human disease, as well as the importance of environmental investigations. Relaying solely on epidemiological investigations would not have revealed the source of infestation at half of the investigations described.
In the field of tick-borne diseases, a newly formed collaboration between the MOE and the MOH is helping us to better understand and control vector-borne diseases.
P064 - Post-covid 19 syndrome versus post-Lyme disease treatment syndrome: are there any similarities?

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Introduction
A significant number of patients report prolonged symptoms following their SARS-COV-2 infection, which have been subsumed under the term of “post COVID 19 syndromes” (PCS). This clinically unspecific syndrome must be put into perspective with other post-infection syndromes like the post treatment syndrome of Lyme disease (PTLD) known for a long time but still little understood and qualified, due to the lack of convincing pathophysiological arguments and a better definition of symptoms related to the initial infection. The magnitude of the disease and the prevalence of PCS has led to intensive and rapid research into its pathophysiology, symptoms, and consequences, which has not been done to date for the other functional post-infection syndromes.

Aims
We aimed to compare the pathophysiological arguments, clinical symptoms, and profiles of patients presenting with SPTC and PTDL to identify similarities that could allow a better characterization of post-infectious syndromes.

Methods
Literature review on Pubmed. For PTLD we included all articles identified with the term “Post treatment lyme diseasease” including adults patients meeting the consensual definition of PTLD. Regarding PCS, given the profusion of publications, we used the terms "long-COVID" OR "post-acute COVID syndrome" OR “persistent COVID-19” then adding the term “pathophysiology” and “symptoms”. Research was limited to review and meta analysis for symptoms, we also include reviews for the pathophysiology.

Results
45/128 and 43/240 articles relating to PTLD and PCS respectively were analysed.
While a consensual definition of PTLD exist since 2006, the definition of PCS is still under debate.
In both pathologies there are still no specific diagnostic tests. We identified the following common inflammatory and immune parameters: elevated levels of CXCL9 and CXCL10 have been reported during the acute phase of infections, but these parameters did not seem to be associated with persistent symptoms; high levels of autoantibodies targeting endothelial cell growth factor (ECGF), apolipoprotein B-100 and metalloproteinase MMP 10 have been observed both in patients with PCS following severe infection and PTLD; increased serum amyloid A protein levels.
The common symptoms found in both syndromes were: fatigue with an impact on quality of life assessed by validated scales, cognitive disorders, depressive syndromes, sleep disorders; arthralgia and myalgia, postural orthostatic tachycardia syndrome.
Regarding patients profils, women were more often represented but age was a source of discordance in both syndromes with studies reporting higher prevalence alternatively in young adults or in older subjects.
Conclusion
We found similar pathophysiological pathway, clinical symptoms, and patient profiles between PCS and PTLD. PCS provides an opportunity to address the complexity of post-infectious functional syndromes, to better understand the mechanisms underlying them and to optimize patient management. Results from studies on patients with PCS syndrome could guide research on PTLD, particularly in terms of physiopathology.
P066 - Borrelia miyamotoi infection negatively impacts pregnancy outcomes in immunodeficient Rag1-/- but not wild-type mice

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Introduction & Aims
Borrelia miyamotoi (B. miyamotoi) is an emerging hard body tick-transmitted relapsing fever (RF) spirochete causing human disease. Soft body tick RF infections can cause adverse pregnancy outcomes in humans and mice; thus, we assessed pregnancy outcomes in B. miyamotoi-infected mice using the clinical isolate CT14D4. As humans and rodents both have a discoid shaped and hemochorial placentae where maternal blood comes in direct contact with the fetal chorion, studies of pregnancy in mice may share direct relevance to human disease.

Methods
Female BALB/cJ+/+ (WT) and C.129S7(B6)-Rag1<sup>tm1Mom</sup>/J (Rag1-/-) mice were inoculated 1 week prior to mating with CT14D4-infected Rag1-/- mouse plasma. Rag1-/- mice were similarly infected 1 day following mating. Complete blood counts, litter size, pup survival and presence of spirochetemia were determined. Rag1-/- mice were also infected by tick transmission of CT14D4 and analyzed at embryonic day (ED) 18.5 or after term delivery.

Results
In multiple experiments when infection occurred one week before mating there was no difference in the survival rate of pups from infected and uninfected Rag1-/- or WT dams. Litter size was variable and independent of maternal infection in all experiments. Pup survival rate from Rag1-/- dams infected post mating, however, was significantly lower than from uninfected dams (6/10 infected dams delivered live pups with all pups from one dam dying after birth whereas 10/10 uninfected Rag1-/- dams delivered live pups). The infection rate of surviving weanling pups was low and sporadic regardless of the timing of B. miyamotoi infection during gestation. Infection of Rag1-/- mice using CT14D4infected ticks prior to mating resulted in both lower pregnancy rates and smaller litter size compared to Rag1-/- dams infested with uninfected ticks. To determine if there was intra-uterine death, pregnant dams were sacrificed at ED18.5. The number of grossly viable fetal-placental units present in infected dams was significantly higher than the number of live pups observed on day 0 postnatally. Spirochetes were demonstrated in the blood by Warthin Starry (WS)-stained tissue sections from the infected Rag1-/- dams. None were detected in the blood or tissue sections of any of the ED18.5 embryos - an indication that infection occurs during or post-delivery. However, spirochetes were found variably within the yolk sac and placenta.

Conclusion
Our results show that hard tick B. miyamotoi infection has adverse effects on pregnancy and fetal viability in Rag1-/- mice but not WT mice. These results differ from soft tick-RF infection, which can lead to intrauterine growth retardation, fetal demise, and congenital infection in WT mice.
Introduction

Tick-borne encephalitis (TBE) caused by the European subtype of TBE virus (TBEV) usually has a biphasic course. It begins with unspecific febrile illness, followed by short improvement and central nervous system (CNS) involvement. Because TBE is not yet suspected during the initial phase, knowledge of early TBE pathogenesis is incomplete.

Aim

The aim of the present study was to assess laboratory and immune findings in a cohort of TBE patients followed longitudinally through the clinical course of TBE from initial viremia (initial phase) through CNS involvement during the second phase of TBE.

Methods

Laboratory and immune findings in the initial and second (meningoencephalitic) phase of TBE were evaluated in 88 well-defined adult patients. To ascertain the distinguishing immune features in the two phases of illness in each compartment (serum, cerebrospinal fluid (CSF)) exploratory modeling of the dataset was performed using sparse Partial Least Squares Regression combined with Discriminant Analysis. Initial phase of TBE was defined by: i) the presence of fever and constitutional symptoms, and ii) demonstration of viral RNA in serum/blood, and iii) the absence of signs/symptoms of CNS involvement at the time of actual illness. TBE was defined by: i) the presence of clinical signs/symptoms of meningitis/meningoencephalitis, and ii) elevated CSF leukocyte counts (>5×10⁶ cells/L), and iii) demonstration of a recent infection with TBEV indicated by serum IgM and IgG antibodies or IgG seroconversion in paired serum samples.

Results

Comparison of 9 blood parameters revealed that laboratory abnormalities, consisting of low total
leukocyte counts, neutropenia, lymphopenia, monocytopenia, thrombocytopenia and increased liver enzymes levels, were predominately associated with the initial phase of TBE and resolved thereafter. Assessment of 29 immune mediators in serum during the initial phase, and in serum and CSF during the second phase of TBE revealed highly distinct clustering patterns among the 3 groups. In the initial phase of TBE the primary finding in serum was a rather heterogeneous immune response involving innate (CXCL11), B cell (CXCL13, BAFF) and T cell mediators (IL-27 and IL-4). During the second phase of TBE, growth factors associated with angiogenesis (GRO-α and VEGF-A) were the predominant characteristic in serum, whereas innate and Th1 mediators were the defining feature of immune responses in CSF.

**Conclusion**

The initial phase of TBE is associated with transitory laboratory abnormalities suggesting bone marrow as well as liver involvement and with rather heterogeneous response involving innate, B cell and T cell mediators. In contrast, the second phase of TBE is characterized with angiogenic immune responses in serum and marked innate and Th1 adaptive immune responses in CSF implying that distinct immune processes play a role in the pathophysiology of different phases of TBE.

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*Borrelia burgdorferi* is the causative agent of Lyme disease. Lyme disease spirochetes from infected *Ixodes* ticks can be transferred to mammalian hosts during tick feeding. There are many different proteins, especially surface localized proteins, which play an important role for the spirochete to resist the immune response, attach to new targets, bind and utilize nutrients, spread and proliferate. *B. burgdorferi* encodes at least 130 lipoproteins, i.e., proteins that are attached covalently to membrane lipids. Although lipoproteins are thought to play a significant role in the infection process the exact function of the majority of lipoproteins is unknown and they do not show homology to proteins with known functions from any other organisms. Our progress in structural characterization of *B. burgdorferi* outer surface proteins, such as BBA64, BBE31, BB0365 and BB0323 has allowed us to learn new details about *B. burgdorferi* at the molecular level. Given that structural research in our laboratory is actively pursuing and new goals are being set, one of the most recent challenges is the research of various previously uncharacterized paralogous families, such as PFam12. The 3D protein structures obtained by x-ray crystallography and functional studies shows very promising initial results to link the role of PFam12 members with the biofilm formation.

Additionally, by using x-ray crystallography we have characterized another *B. burgdorferi* protein BB0789 that is known to be essential for mouse and tick infectivity and *in vitro* growth of *B. burgdorferi*. The structural analysis revealed the AAA+ ATPase and the zinc-dependent metalloprotease domains arranged in a hexamer that is essential for ATPase and proteolitic activity and we confirmed that BB0789 is functionally active protease capable of hydrolyzing ATP thus providing novel structural-functional insights into the protein that is known to be absolutely necessary for *B. burgdorferi* to cause Lyme disease.

This work was supported by the LZP grant Nr. lzp-2021/1-0068.
P069 - A Murine Model of Lyme Disease Demonstrates That Borrelia burgdorferi Colonizes the Dura Mater and Induces Inflammation in the Central Nervous System

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Introduction
Lyme borreliosis, which is caused by infection with Borrelia burgdorferi and related species, can lead to inflammatory pathologies affecting the joints, heart, and nervous systems including the central nervous system (CNS). Inbred laboratory mice have been used to define the kinetics of B. burgdorferi infection and host immune responses in joints and heart, however similar studies are lacking in the CNS of these animals.

Objections/Aims
A tractable animal model for investigating host-Borrelia interactions in the CNS is key to understanding the mechanisms of CNS pathogenesis. Therefore, we characterized the kinetics of B. burgdorferi colonization and associated immune responses in the CNS of mice during early and subacute infection.

Methods
Fluorescence-immunohistochemistry, intravital microscopy, bacterial culture, and quantitative polymerase chain reaction (PCR) were used to examine colonization of the dura mater by B. burgdorferi. RNA-sequencing and quantitative reverse transcriptase-PCR were used to determine changes in host gene expression. Tissues including dura mater and cortex were fixed and stained with hematoxylin and eosin for histopatholgical analysis.

Results
We found B. burgdorferi routinely colonized the dura mater of C3H mice, with peak spirochete burden at day 7 post-infection. Dura mater colonization was observed for several Lyme disease agents including B. burgdorferi, B. garinii, and B. mayonii. B. burgdorferi infection was associated with increased expression of inflammatory cytokines and a robust interferon (IFN) response in the dura mater. Histopathologic changes including leukocytic infiltrates and vascular changes were also observed in the meninges of infected animals. In contrast to the meninges, we did not detect B. burgdorferi, infiltrating leukocytes, or large-scale changes in cytokine profiles in the cerebral cortex or hippocampus during infection; however, both brain regions demonstrated similar changes in expression of IFN-stimulated genes as observed in peripheral tissues and meninges.

Conclusions
Taken together, B. burgdorferi is capable of colonizing the meninges in laboratory mice, and induces localized inflammation similar to peripheral tissues. A sterile IFN response in the absence of B. burgdorferi or inflammatory cytokines is unique to the brain parenchyma, and provides insight into the potential mechanisms of CNS pathology associated with this important pathogen.
P070 - Borrelia burgdorferi Gac protein binds specifically to DNA adjacent to the ospC promoter

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Introduction

Borrelia burgdorferi requires the production of the outer surface protein OspC for the establishment of mammalian infection. Bacteria within an unfed tick do not produce OspC. When the tick takes its blood meal, production of OspC increases and it continues until the early stages of mammalian infection. Later stages of mammalian infection, expression of OspC needs to be shut off, otherwise antibodies that are made against OspC will cause the bacteria to be cleared off the body. Tightly regulation of OspC is very important, as bacteria that do not produce the protein or make too much of it, is unable to successfully colonize a mammalian host. The mechanism that controls OspC regulation is not fully understood. Better understanding of the regulation that controls OspC expression could provide insight into development of new therapeutics.

Methods

DNA affinity chromatography was performed with cytoplasmic extract from the B31 strain of B. burgdorferi to identify proteins that bind to a region of the ospC promoter. The pulled down protein was identified through mass spectrometry as the C-terminal domain of the GyrA protein, also known as Gac. Electrophoretic mobility shift assays (EMSAs) were performed with recombinant Gac protein to test for DNA binding to an IRD labeled probe of the 5’ region of the ospC promoter. Additional EMSAs were done to test for DNA binding affinity and specificity. A combination of qRT-PCR and western blotting was performed on B. burgdorferi lysates generated from the wildtype strain and a Gac deficient strain, CKO1, to identify the effects of Gac on ospC at the transcriptional and translational levels.

Results

The C-terminus of GyrA (Gac) was found to bind with high affinity to the 5′ region of the ospC promoter. The combination of qRT-PCR and western blotting in B. burgdorferi lysates from the wildtype strain and CKO1 strain exhibited similar results at both the transcript and protein levels by displaying higher levels of OspC in the CKO1 strain in comparison to the wildtype strain.

Conclusion

Gac binds with high affinity to the 5′ region of ospC and functions as a transcriptional repressor of ospC.
P071- Elucidating the interactions between NapA-peptidoglycan and implications for the immune response

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Introduction

* Borrelia burgdorferi*, the causative agent of Lyme disease, has characteristics that result in unusual interaction(s) with the human host compared to other bacterial pathogens. It lacks classical virulence factors and undergoes antigenic variation; two features that have hindered both diagnostic and vaccine development. In order to address these challenges, we have been investigating the peptidoglycan (PG) of *B. burgdorferi*. Since this bacterium lacks a PG recycling pathway, it consistently sheds a significant amount of PG into the host environment during infection. The PG of *B. burgdorferi* persists in the synovial fluid of Lyme arthritis patients, and these same patients produce long-lasting antibodies that are specific to the Lyme spirochete’s cell wall. Recently, Neutrophil Attracting Protein A (NapA) has been discovered to be a PG-associated protein that plays an important role in protection against cell wall stress as well as modulation of the host immune response.

Aims

We propose that the association between the two immunomodulatory molecules—NapA and PG—have implications for not only host immune response(s) during infection, but for Lyme disease diagnostic and vaccine development.

Methods/Results

Here, we used a flow cytometry-based strategy known as Saccuflow to characterize the association between NapA and the *B. burgdorferi* sacculus. We then exploited this interaction to assess both the vaccine and diagnostic potential of covalently crosslinked NapA-PG antigens in live mice.

Conclusion

Our findings provide new insights into the pathogenicity of NapA associated PG, and promising avenues to exploit this relationship as a means to diagnose and prevent Lyme disease.
The skin plays a key role at different stages of Lyme borreliosis. In the early stage, the process of *Borrelia* transmission involves several actors including *Ixodes* tick saliva, the bacterium and numerous effectors of the host immune system. However, there is still limited knowledge on the potential role of the microbiota present at the skin interface. Different works have shown that the skin microbiota can modulate the inflammatory response to an infectious agent. It could constitute a key element in early response to pathogens. This work aims to study how the secretome of three major commensal bacteria of the skin microbiota (*Staphylococcus epidermidis*, *Cutibacterium acnes* and *Corynebacterium striatum*) impacts the inflammation induced by *Borrelia*. For this purpose, we analyzed the immune signaling of human primary fibroblasts and keratinocytes, the two main skin-resident cells, co-incubated in vitro with the two types of bacteria. The secretion of IL-8 and TNF-α, and the induction of IL-8, SOD2, MCP-1, CXCL1 and MMP-3 genes were studied by ELISA and qRT-PCR, respectively, after co-stimulation of the skin cells by bacterial secretomes and *B. burgdorferi* s.s. N40. In addition, the expression of the antimicrobial peptide, human β-Defensin 2 (hBD2) gene, by keratinocytes was also determined.

The impact of the secretomes differs depending on the bacterial species and skin cell types. Stimulation of human keratinocytes with *B. burgdorferi* s.s. N40 alone increases IL-8 and hBD2 gene expression. Addition of *S. epidermidis*, *C. striatum* and *C. acnes* secretomes tends to reduce the IL-8 gene expression in presence of *Borrelia*. *C. acnes* secretome alone increases IL-8 in absence of *Borrelia*. These three secretomes also decrease the expression of hBD2 gene during co-stimulation. No particular effects were observed for TNF-α secretion and SOD2, MCP-1, CXCL1 and MMP-3 gene expression.

*Borrelia* alone increases IL-8, SOD2, CXCL1, MCP-1 gene expression and IL-8 secretion of fibroblasts. The secretome of *S. epidermidis* alone induces IL-8 secretion and potentiates IL-8 and SOD2 gene expressions at all concentrations tested, and above certain concentration threshold, CXCL1 and MCP-1 genes were induced during co-stimulation with *Borrelia*. The secretome of *C. acnes* potentiates the expression of SOD2 and CXCL1 genes at high concentrations and decreases the expression of MMP-3 at low concentration. *C. striatum* secretome and *Borrelia* synergize the induction of CXCL1 expression at high concentrations (15 µg/mL) and potentiate MCP-1 gene expression in a dose-dependent manner. No particular effects were observed for TNF-α secretion and MMP-3 gene expression.

In conclusion, skin commensal bacteria can modulate the inflammatory response of skin resident cells but the analysis of the molecular mechanisms as well as the clinical implications of this modulation remain to be determined.
Borrelia burgdorferi, the causative agent of Lyme disease, is an extreme auxotroph. The lack of biosynthetic capabilities is consistent with its reductive genome, and the spirochete must scavenge a large array of nutrients from its vector and host environments, thereby intimately linking bacterial physiology with pathogenesis. While B. burgdorferi’s requirements for a number of substrates (e.g., carbohydrates, fatty acids) have been well studied, only recently was it demonstrated that amino acid (AA) acquisition is primarily achieved via peptide uptake.

Our previous studies have shown that B. burgdorferi is fully dependent on peptide uptake through its oligopeptide transport (Opp) system for viability, an attribute that is, so far, unique to this pathogen. As such, the Opp system presents an intriguing and highly specific point of therapeutic intervention. Peptide transport through the Opp system relies upon five different spatiotemporally expressed binding proteins (OppA1-5).

To date, we have evaluated the independent roles of OppA1, OppA2, and OppA5 throughout the enzootic cycle. Each OppA mutant has produced distinctive phenotypes that are uniquely orient to specific stages of the cycle. The loss of OppA1 renders spirochetes sensitive to cell lysis during tick acquisition, a phenotype suggesting osmosensitivity. Ablation of OppA2 expression results in spirochetes that are unable to hematogenously disseminate within the mammal, restricting the spirochetes to subcutaneous dissemination and skin colonization. Finally, OppA5 mutants are unable to persist long term within mammals, particularly in the heart and joints. While we continue characterization of individual OppA3 and OppA4 mutants, we also plan to evaluate double mutants, specifically an OppA1/OppA2 mutant, to discover the “minimal” intervention required to render the spirochete non-viable.

Further, we have developed a multi-pronged approach to evaluate the structural and functional characteristics of OppA1-5 and their potential for inhibitor development. Using in silico molecular dynamic studies, we will evaluate each binding protein’s ability to bind combinatorial peptide substrates. These results are then tested by a high throughput thermal shift assay to confirm in silico results and modify docking parameters within our system. Ligands will also be assessed by an Escherichia coli heterologous Opp expression system to confirm ligand transport. Selected ligands will further undergo a more detailed analysis by isothermal calorimetry and surface plasma resonance to define binding. These structural and liganding studies will further inform drug design approached to either 1) block peptide transport through the Opp system or 2) target bactericidal compound transport via the Opp system. Potential inhibitors will be screened by in silico modeling, in vitro cultivation, and finally, by in vivo infection models.

We believe that the development of a highly targeted therapeutic intervention for B. burgdorferi, centered around its core requirements for AA would significantly impact the global approach to clinical treatment.
P074 - P66 is a bacterial checkpoint mimic of mammalian “don’t eat me” signal CD47 and facilitates macrophage evasion by *Borrelia burgdorferi*.

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**Introduction**

In the co-evolution of host and pathogen, pathogens developed mechanisms to dampen and evade phagocytic clearance. Pathogen infected cells and cancer cells hijack the CD47-SIRPα axis by upregulating CD47 expression to evade macrophage surveillance. We hypothesized that, similar to poxviruses, which are known to conserve a mimic of mammalian CD47, bacteria may also inhibit innate immunity through mimicry. We reasoned that bacterial mimics of the mammalian ligand CD47 would allow bacteria to avoid a macrophage-mediated innate immune response and therefore assist in the establishment and persistence of infection. Like poxviruses, the bacterial spirochete *Borrelia burgdorferi* (*B. burgdorferi*) is adept at evading innate immune clearance. Because *B. burgdorferi* can establish a persistent infection, we hypothesized that 1) *B. burgdorferi* have a mechanism of immune evasion by inhibiting components of the human immune system and 2) *B. burgdorferi* produce a mimic of the mammalian surface protein CD47.

**Methods**

Fluorescence-Activated Cell Sorting (FACS) of *B. burgdorferi* stained for CV1G4, B6H12, and MIAP410. IgM binding to serum from B6 mice infected with *B. burgdorferi* at clearable and non-clearable bacterial loads either pre-treated with CV1G4, IgG4, or no treatment. *B. burgdorferi* stained either positively or negatively for CV1G4 were lysed under non-denaturing conditions and lysate was subjected to CV1G4 or IgG4 enrichment prior to SDS-PAGE. SDS-PAGE gel bands of interest were excised and subjected in-gel trypsin digestion followed by mass spectrometry analysis. Immunoprecipitation of recombinant P66 enriched by CV1G4, IgG4, or SIRPα. Biacore affinity measurements between P66 and CV1G4 or SIRPα. Time-lapse live-cell-microscopy-based phagocytosis assays using human monocyte derived macrophages and either WT or Δp66 *B. burgdorferi* at an MOI of 10.

**Results**

*B. burgdorferi* express a surface protein that binds to a CD47 affinity reagent and alters the course of infection. P66 binds CV1-G4 and SIRPα. Δp66 *B. burgdorferi* are more-readyly phagocytosed by human macrophages compared to WT as measured by pHrodo-positive phagocytic events at an MOI of 10. P66 elicits an immune response in individuals with less-severe symptoms and those who return to health.

**Conclusion**

This is the first report of a bacterial protein signaling through a mammalian “don’t eat me” receptor in which *B. burgdorferi* express a CD47-like “don’t eat me” signal ligand, P66, and that loss of p66 increases *B. burgdorferi* clearance by macrophages. Other bacterial proteins likely also bind SIRPα and play a role in co-evolution. Furthermore, SIRPα polymorphisms may have broad-reaching implications for phagocytic capacity. We postulate that if bacteria can evade phagocytic clearance, a course of bacteriostatic antibiotics may not be sufficient for all variants
of SIRPα to clear persistent bacterial infection. Blockade of the P66-SIRPα checkpoint pathway may have therapeutic potential as a supportive immunotherapy.
Introduction

Lyme borreliosis (LB) is a complex inflammatory disorder caused by spirochetes in the Borrelia burgdorferi sensu lato (s.l.)-complex which may be transmitted to humans by Ixodes ticks. The outcome following infection differs between individuals. Asymptomatic seroconversion is common. Borrelia infection can also cause clinical symptoms ranging from a local skin rash to disseminated disease typically involving skin, joints, or nervous system. The mechanisms behind the variable clinical outcomes are far from fully understood, but the establishment of an optimal immune response against Borrelia is believed to be of crucial importance for resolution of the disease. Previous studies have suggested that single nucleotide polymorphisms (SNP) in immune-related genes encoding Toll-like receptor (TLR)-1, TLR-2, interleukin (IL)-23 receptor (R) and anoctamin (ANO10) in macrophages may have an impact on the course of LB.

Aims

The aim of the study was to investigate if an association between symptom development and five previously identified SNP could be demonstrated in tick-bitten individuals infected by B. burgdorferi s.l.

Methods

Acute and three-month convalescent serum samples from tick-bitten participants in the TBD STING Study (n= approximately 5,500) were analysed for specific IgG antibodies against Borrelia. A total of 211 study participants showed seroconversion, and whole blood samples were available from 208 of them. DNA was extracted from EDTA blood using the instrument EZ1 Advanced XL together with the EZ1&2 Blood Kits. The following SNP were analysed using TaqMan SNP Genotyping Assays: TLR-1 (Rs5743618), TLR-2 (Rs5743708), IL-23R (Rs11209026), ANO10-ZNF821 (Rs17850869), and ANO10-R263H (Rs41289586). The frequencies of the different alleles in individuals with asymptomatic seroconversion (n=140) were compared with individuals who reported symptoms indicative of LB (n=68) using chi-square analysis.

Results

The median age of the included individuals was 64 years and 60% were female. There were no significant differences between the two groups regarding age or gender distribution. The allele frequencies in the TLR-1, TLR-2, IL-23R, ANO10-ZNF821, ANO10-R263H, and IL-23R found in this study were comparable to what has previously been reported for a European population. No significant differences were found between the asymptomatic and the symptomatic group regarding the allele frequencies in any of the studied SNP.

Conclusion

Previous studies, mainly performed in vitro, have indicated that certain SNP in the genes encoding TLR-1, TLR-2, IL-23R, ANO10- ZNF821, and ANO10-R263H are of importance for the human immune response against B. burgdorferi s.l. However, in this study including a limited number of tick-bitten individuals with demonstrated seroconversion, we could not confirm that
these SNP have an impact on development of symptoms in humans upon infection. Under natural circumstances, a multitude of variables are likely to influence the clinical course after transmission of the *Borrelia* spirochetes, varying degree of virulence in different *B. burgdorferi* s.l. species and strains being one such factor.

**P076 - Borrelia burgdorferi** DnaA and EbfC regulate production of the DNA polymerase III holoenzyme and EbfC

*Mr. Andrew Krusenstjerna*

EbfC is a highly conserved nucleoid-associated (‘histone-like’) protein that binds to specific sites throughout the *Borrelia burgdorferi* genome. Dysregulation of EbfC has global effects on the levels of borrelial transcript and protein. EbfC plays a key role in the regulation of the Erp outer surface proteins, which adhere to vertebrate host innate immune serum proteins and extracellular-matrix components. The *ebfC* gene is in a bicistronic operon with *dnaX*, which encodes the Tau subunit of the DNA polymerase III holoenzyme. Transcript levels of both *dnaX* and *ebfC* are highest when *B. burgdorferi* enters exponential growth and decreases during stationary phase. DnaA, the master initiator of DNA replication, was found to bind specifically within the unusually long 179bp 5' UTR DNA of the *dnaX-ebfC* operon. EbfC was also found to bind two sites in the 5' UTR DNA and to one site in the RNA. Surprisingly, while DnaA alone promotes the *in vitro* expression of the *dnaX-ebfC* operon, the addition of EbfC abrogates this effect. Thus, DnaA regulates levels of at least one subunit of DNA polymerase III, a nucleoid-associated protein, and, through EbfC, the Erps. In essence, it appears *B. burgdorferi* has evolved a means to coordinate DNA replication with adaptation to its host.
P077 - Structural evolution of an immune evasion determinant shapes Lyme borreliae host tropism

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Introduction
Host-pathogen interactions often lead to host tropism, pathogens’ preferential survival in particular hosts. Maintained mainly by rodents and birds, B. burgdorferi (Bb), the causative agent of Lyme disease (LD), is an ideal system to study host tropism.

Objections/Aims
Herein, we tested the concept that host tropism among Bb strains can be attributed to polymorphic Bb proteins promoting evasion to complement, an innate immune defense in vertebrate blood.

Methods and Results
We grouped 20 Bb strains into three distinct phenotypes based on complement-dependent, rodent- or bird-specific sera survivability and/or infectivity. We analyzed phylogenomics and pinpointed OspC and CspZ as potential host tropism drivers. We further found that CspZ binds to a rodent and/or bird complement inhibitor, FH, to inactivate complement in an allelic-specific manner. Through our recently solved high-resolution structure of CspZ-FH, we found that despite ~98% identity, CspZ harbors a short variable loop structure within the CspZ-FH-binding interface. Swapping loops between rodent- and bird-associated CspZ variants altered these variants’ ability to promote host-specific complement inactivation and early-onset dissemination, defining that loop as Bb host tropism determinant. Finally, we linked these loops and respective host-specific phenotypes to distinct CspZ phylogenetic lineages spanning 174 Bb isolates from Northern hemisphere, illustrating the evolutionary history of CspZ-driven host tropism

Conclusions
This multi-disciplinary study demonstrates how a structurally variable motif from an immune evasion determinant shapes host tropism.
Fibroblast-like synoviocytes activate natural killer cells and shape inflammation and wound healing in Lyme arthritis

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Background
Lyme arthritis (LA) is caused by infection with Borrelia burgdorferi (Bb). Despite proper antibiotic treatment, some individuals can develop proliferative synovitis that persists for months or years after infection is cleared, a condition known as post-infectious LA. Fibroblast-like synoviocytes (FLS) are the most abundant cell type found in the synovial lesion and are critical in immune responses to Bb during infection and wound healing post-infection. Previous studies have shown that natural killer (NK) cells are important sources of interferon-gamma (IFNγ) in synovial tissue and synovial fluid of LA patients and are potent activators of FLS. However, the cellular mechanisms of FLS-NK cell interactions remain incompletely understood.

Methods
FLS were cultured from joints of uninfected and Bb-infected C57BL/6 (B6) mice and stimulated in vitro with IFNγ, Bb, and/or peptidoglycan (PG). Flow cytometry, gene expression analysis, and cytokine profiling were used to characterize immune activation. FLS were co-cultured with NK cells, and NK cell activation was assessed using a proliferation assay and by measuring cytokine secretion. Cell migration assays were performed to assess FLS wound healing responses. Similar experiments were performed using FLS cultured from synovial tissue of patients with LA, rheumatoid arthritis (RA), or osteoarthritis (OA), and stimulated under similar conditions.

Results
When treated with IFNγ, FLS were hyper-responsive to Bb and produced high levels of inflammatory mediators, including IL-15/IL-15Rα, which induces NK cell activation and proliferation. FLS pre-treated with PG + IFNγ induced marked NK cell proliferation and secretion of high levels of pro-inflammatory cytokines, including IFNγ, IL-6, IL-17, and IL-27. PG alone induced similar levels of IL-6, IL-17, and IL-27, but not IFNγ. Moreover, naïve B6 FLS treated with IFNγ or Bb + IFNγ displayed significantly reduced wound healing compared to unstimulated FLS or Bb alone. In FLS from 4 week-infected mice, stimulation with Bb alone reduced wound healing to a higher degree compared to similar conditions in FLS from naïve mice. In human FLS, inflammatory and wound healing phenotypes varied dramatically depending on the patient diagnosis (LA, RA, or OA).

Conclusions
Our data indicate that FLS-NK cell interactions drive inflammation and block appropriate repair of damaged tissue. This may be occurring through two related pathways. First, FLS are directly activated by Bb and/or IFNγ produced by NK cells, which downregulates FLS wound healing responses. Second, FLS activate NK cells, which produce pro-inflammatory cytokines and cause cellular and tissue damage. Further studies will examine whether NK cells target and kill FLS and other stromal cells and will determine epigenetic changes to FLS during immune responses to infection.
Peptidoglycan acts as an immune adjuvant to enhance CD4+ T cell activation by MHC-II+ fibroblast-like synoviocytes

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**Introduction**

Post-infectious Lyme arthritis (LA) is characterized by proliferative synovitis accompanied by dysregulated autoimmune Th1/IFN-ɣ responses and MHC-II+ expression in fibroblast-like synoviocytes (FLS), a common cell type in inflamed joints. High levels of *B. burgdorferi* cell wall peptidoglycan (PG) have been identified in inflamed joints of post-infectious LA patients. However, the effects of *B. burgdorferi* PG on innate and adaptive immune responses are not well characterized. We hypothesize that during LA, *B. burgdorferi* PG acts as an adjuvant to enhance antigen presentation and autoimmune-like CD4+ T cell activation in the joint.

**Methods**

Primary murine macrophages and FLS, both abundant cell types in LA synovial lesions, were stimulated *in vitro* with IFN-ɣ, *B. burgdorferi* PG, or both. Immune effector responses were measured by surface expression of MHC-II and production of proinflammatory mediators. To determine the effects on T cell responses, IFN-ɣ-primed MHC-II+ B6 FLS were co-cultured with OT-II mouse T cells (genetically modified to have identical antigen-specific T cell receptors) and supplemented with the cognate OVA323-339 peptide. An irrelevant OVA257-264 peptide, as well as FLS lacking the PG receptor NOD2 were used as negative controls. T cell responses were measured by flow cytometry and multiplex immunoassay.

**Results**

Stimulation of macrophages, a professional antigen presenting cell (APC) type led to upregulation of MHC-II (I-Ab in mice) in an IFN-ɣ-dependent manner, as expected. Stimulation of FLS with IFN-ɣ led to upregulation of MHC-II, consistent with previous *ex vivo* findings, and this response was enhanced by PG stimulation. FLS stimulated with the muramyl-dipeptide portion of PG, a known NOD2 ligand, upregulated production of proinflammatory cytokines IL-1β, IL-6, IL-12, and TNF-α. T cell co-culture with FLS primed with IFN-ɣ induced naive OT-II mouse CD4+ T cell proliferation, with a three-fold increase compared to the unstimulated control. This response was enhanced to a five-fold increase when co-cultured with FLS primed with IFN-ɣ and stimulated with PG. T cell supernatants contained high levels of IFN-ɣ and IL-12 production, indicating a Th1 response profile. Negative controls using irrelevant OVA peptide or NOD2-deficient FLS failed to induce T cell proliferation.

**Conclusions**

These data show that PG acts as an immune adjuvant by enhancing the ability of MHC-II+ FLS to activate MHC-II peptide-specific CD4+ T cell proliferation. This response is similar to autoimmune Th1/IFN-ɣ responses characteristic in LA patients, highlighting the effects of PG on enhanced antigen presentation and autoimmune T cell responses during disease development. Current experiments include investigating PG as an autoimmune adjuvant *in vivo*. This study helps us understand persistent inflammatory responses associated with *B. burgdorferi* PG, even in the absence of live spirochete infection, as well as other chronic inflammatory and autoimmune diseases where infection is thought to be a trigger.
Uncovering the factors modulating gene expression in *Borrelia* is crucial for understanding the pathogenesis of *Borrelia* and thus for the fight against the Lyme disease. Since the interface between *Borrelia* and their environment is the outer membrane, proteins localized on the outer surface must play an important role in transmission. It has previously been shown that the expression of outer surface proteins can be stimulated by temperature, or tick feeding. However, little is known about what exactly causes these changes in expression during tick feeding. Here, we show the effect of temperature and different nutrients on expression of outer surface proteins with a special focus on OspC, the major virulence factor. Infected nymphs were stimulated by membrane feeding, or microinjections, gene expression was measured using RT-PCR, and infectivity was tested in a mouse model. Prior to feeding, *Borrelia* spirochetes in the tick gut showed low expression of OspC and were not infectious. Our results suggest that the expression of OspC is increased when infected ticks are fed or injected with sugar solutions. We show that even a small amount of particular sugars passing through the tick gut can lead to a huge increase in OspC expression. Moreover, gut homogenates prepared from unfed, sugar stimulated nymphs can induce infection. In contrast, we observed no effect of other nutrients on OspC expression. Based on our data, we assume that, in addition to temperature, the main trigger of OspC expression and infectivity is the presence of sugars in the tick gut. However, the tick feeding process itself or the presence of blood might be beneficial, but not crucial.
P083 - Unraveling the role of plasmid topology and cis-acting elements of vlsE in evasion of the host immune response by the Lyme disease spirochete

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Introduction
VlsE antigenic variation in *Borrelia burgdorferi* is a key component of the defense strategy employed by the spirochete to successfully evade host immune surveillance. However, not much is known about this mechanism since genetic manipulation of the distinct well conserved *cis*-acting elements has been challenging. One of the unique features of the spirochete is its segmented genome containing numerous linear and circular plasmids. The *vls* locus resides on one of the linear plasmids, lp28-1, and the expression site of this locus, *vlsE*, harbors a variable region that is flanked on both sides by 17bp direct repeats (DR). Till now, it was considered that *vlsE* recombination occurs only in *cis*. In this study, a *trans* wild type or mutant copy of *vlsE* was carried on either a linear or circular shuttle vector along with the native lp28-1 to assess the ability to undergo recombination. We show for the first time that recombination can occur in *trans* and demonstrate a requirement of the DRs for transcription of *vlsE* which is critical for persistent infection in the host by the pathogen.

Methods
Clones were generated having either wild type or mutant versions of the DR regions of *vlsE* along with its native promoter harbored on a circular or linear pBSV2 vector and transformed into *B. burgdorferi* cells. Groups of C3H mice were infected with the generated strains via needle inoculation, and skin, heart, and bladder tissues were harvested for culture in BSK-II media. Transcript and protein levels were analyzed by qRT-PCR and Western blot respectively.

Results
A direct comparison between linear and circular vectors carrying *vlsE* showed long-term, persistent infection of mice occurs only when *vlsE* is carried on linear shuttle vector. All mice infected with spirochetes harboring a *vlsE* copy on a circular plasmid were cleared prior to four-weeks post infection. Analysis of recovered clones found that the *vlsE* copy did not undergo recombination, suggesting that clearance was due to expression of non-variable VlsE that was likely targeted by host antibodies. Four out of six mice infected with spirochetes harboring the *vlsE* copy on a linear plasmid were not cleared of infection by four weeks post inoculation. DNA sequence analysis showed that the *vlsE* gene copy on the linear plasmid had undergone gene conversion. Mutations in DR regions significantly reduced transcript levels of *vlsE* in vitro. However, the expression of VlsE protein was found to be elevated in the mutant clones compared to the wild type.

Conclusion
Plasmid topology plays an important role for *vlsE* gene conversion. Recombination can occur in *trans* when *vlsE* is present on linear vector. Mutations in direct repeat regions indicate that the 17bp DRs maybe required for *vlsE* transcription. VlsE protein levels do not necessarily correlate with *vlsE* mRNA levels, suggesting that bacterial protein levels may be partially controlled through a posttranscriptional mechanism that can have effects on spirochete’s physiology and/or pathogenesis.
The consistent tick-vertebrate infectious cycle of the Lyme disease spirochete enables Borrelia burgdorferi to control protein expression by monitoring its physiological status

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The Lyme disease spirochete, Borrelia burgdorferi, persists in nature by alternatingly cycling between ticks and vertebrates. During each stage of the infectious cycle, B. burgdorferi produces surface proteins that are necessary for interactions with the tick or vertebrate tissues it encounters, while also repressing the synthesis of unnecessary proteins. Among these are the Erp surface proteins, which are produced during vertebrate infection for interactions with host plasmin, laminin, glycosaminoglycans, and components of the complement system. Erp proteins are not expressed during tick colonization, but are induced when the tick begins to ingest blood from a vertebrate host, a time when the bacteria undergo rapid growth and division. Using the erp genes as a model of borrelian gene regulation, our research group has identified three novel DNA-binding proteins that interact with DNA to control erp transcription. At least two of those regulators are, in turn, affected by DnaA, the master regulator of chromosome replication. Our data indicate that B. burgdorferi has evolved to detect the change from slow to rapid replication during tick feeding as a signal to begin expression of Erp and other vertebrate-specific proteins. The majority of other known regulatory factors of B. burgdorferi also respond to metabolic cues. These observations lead to a model in which the Lyme spirochete recognizes unique environmental conditions encountered during the infectious cycle to “know” where they are and adapt accordingly.
Genomic analysis of B. burgdorferi isolates from patients in distinct geographical locations reveals deep population structure and extensive recombination

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Introduction

Borrelia burgdorferi sensu stricto (Bbss) causes nearly all cases of Lyme disease in North America and a minority of cases in Europe. Previous targeted genotyping systems (e.g., RST, OspC, and MLST) revealed that genetic differences between strains are potentially linked to differences in clinical presentation. However, the extent of this linkage and specific Bbss genes and/or loci involved are not well understood at least in part because only a few human isolates with known clinical information have been sequenced. Here, we report a whole genome sequence (WGS) analysis of a large cohort of patient-derived Bbss isolates collected over three decades at three distinct geographical locations; Northeastern United States, Midwestern United States, and Central Europe.

Materials and Methods

In total, 299 Bbss isolates derived from patients were included in this study; 202 from Northeastern US, and 61 from Midwestern US, and 36 from Slovenia. Isolates were derived from skin or blood by culturing in MKP or BSK medium. Only low passage isolates (passage <5) were used.

Results

The analysis reveals several features of Bbss evolution: First, human isolates of B. burgdorferi possess a core genome consisting of 795 genes. These core genes are shared by >95% of all isolates and can be used to infer WGS phylogeny. Second, WGS phylogenies are concordant with MLST type and correlate strongly with OspC type and RST type. The discrepancies in these associations revealed that RST typing is polyphyletic and that OspC phylogenies are shaped by extensive recombination at the OspC locus, a phenomenon which does not affect the accessory genome as a whole. Finally, the subclade structure of WGS phylogenies reveals at least two intercontinental migration events between Slovenia and the US, and at least two migration events within the US between the Northeast and upper Midwest.

Conclusion

This initial large-scale analysis provides insight into the origin and evolutionary history of Bbss and lays a foundation for future microbial genetic association studies to link the clinical presentation and possibly the outcome of Lyme disease with specific Bbss loci.
P086 - Systemic Immune Responses in Patients with Early Borrelia afzelii Lyme Borreliosis

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Introduction
Data on the association between patient age and presentation of early Lyme borreliosis (LB) and host immune responses in early LB are limited. We aimed to investigate the association between age and systemic innate, Th1 and Th17 immune responses in patients with skin culture-positive Borrelia afzelii erythema migrans (EM) and to explore the association between markers of serum immune responses and LB-associated symptoms.

Methods
Fifteen cytokine and chemokine levels, representative of innate, Th1, and Th17 immune responses, were assessed using a bead-based Luminex multiplex assay in acute sera from 96 adult patients with skin culture-positive B. afzelii EM, selected randomly from 1220 patients enrolled prospectively in several clinical trials from 2006 to 2018 at the University Medical Centre Ljubljana, Slovenia; 48 patients were aged ≥ 50 years and 48 patients were < 50 years old.

Results
Mean serum cytokine and chemokine levels were low. When taking into account the proportion of patients with cytokine or chemokine concentrations below the lowest detectable limit and adjusting analysis for multiple comparisons, only levels of IL-23 (p=0.033), representative of the Th17 immune response, differed between the two age groups of patients with higher concentrations detected in younger patients. In addition, we did not find differences in systemic inflammatory responses when comparing patients with and without LB-associated symptoms at enrollment.

Conclusion
In our study of European patients with EM, no significant differences in systemic immune responses represented by selected cytokines and chemokines in serum samples of patients infected with B. afzelii suggest that systemic mediators are not pivotal drivers of age-related differences in the clinical presentation of early LB or LB-associated symptoms. Localized immune responses in the skin and/or other pathogenetic mechanisms may be more important in this regard.
P087 - Detection of *Borrelia burgdorferi* in non-invasive specimens during early infection in a mouse model

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**Introduction**

*Borrelia burgdorferi* sensu lato, the causative agent of Lyme borreliosis (LB), is a vector-borne bacterial infection that is transmitted through the bite of an infected tick. The use of non-invasive specimens in both human and canine LB diagnosis is desirable. Here we investigated the presence of *B. burgdorferi* in different specimen samples collected at different time points after mouse inoculation.

**Methods**

Ten female C3H/HeN mice at 6 weeks of age were used for this study. On Day 0, each mouse was intradermally inoculated with \(10^6\) spirochetes (*B. burgdorferi* sensu stricto B31) into the dorsum. Multiple samples such as urines, whole blood, ear biopsies were collected on Day 2, 4, 6, 8, 10, 14, 16, 18, 21, 28 and 35. Saliva was only collected on Day 14 using sterile buccal swabs. Furthermore, three to four mice were euthanized on Day 18, 28 and 35 and tissue samples including heart, meninges, cerebral cortex, liver, kidney, spleen, joint, ear biopsies, salivary gland were collected and screened for the presence of *B. burgdorferi* using nested PCR and/or culture.

**Results**

*Borrelia burgdorferi* DNA was early detected in blood and urine samples (Day 2). Even though this detection was transient, it remained possible in blood and urine until Day 8 and 21 respectively. Interestingly, *B. burgdorferi* DNA from buccal swabs was detected in 5 mice (50%) on Day 14. *Post-mortem* analysis showed dissemination and persistence of spirochetes in almost all organs except cerebral cortex (no *B. burgdorferi* DNA detection). All salivary glands were positive for *Borrelia* in PCR and at least one in culture. Finally, we found that all meninges were consistently culture positive for spirochetes.

**Conclusion**
Even though relatively small in terms of numbers, our result reports a new insight about the detection/diagnosis of *B. burgdorferi* using non-invasive biological samples.
P088 - Serum resistance and protein profile determination of *Borrelia burgdorferi* sensu lato strains from Serbia

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**Introduction**

Lyme borreliosis (LB) is an infectious tick-borne disease caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex. The pathogenicity of borrelia depends on their invasiveness, antigenic variability, lymphocyte stimulation, and resistance to host complement. Despite the effectiveness and abundance of complement, *Borrelia* can overcome defense mechanisms.

**Aims**

Since information on the exact species that cause LB in Serbia is lacking, and on the pathogenic potential of *Borrelia lusitaniae* (predominant species in Serbia), we were interested in the susceptibility of local borrelia strains to human complement. Additionally, the objective of our study was to analyze the protein profiles (OspA, OspB, and OspC) of strains and assess the correlation between expressed proteins in borrelia and their resistance to normal human serum (NHS) to clarify the pathogenic potential, *in vitro*.

**Methods**

The 27 local borrelia strains (*Borrelia afzelii*, *Borrelia bavariensis*, *Borrelia garinii*, *Borrelia valaisiana* and *B. lusitaniae*) isolated from *Ixodes ricinus* ticks and four *B. lusitaniae* from Spain and Portugal (three isolated from ticks and one from human skin) were included in the study. The direct killing assay was performed to investigate the susceptibility of *Borrelia* to NHS. Loss of motility and extent of bleb formation of spirochetes after three hours of incubation, in NHS versus heat-inactivated serum, was indicative of complement-mediated killing and inactivation of spirochetes. For protein profile characterization, electrophoreses were performed for every
isolate, and molecular masses of proteins were determined according to their position in the gel compared to molecular mass standards.

Results
The presence of OspA, OspB, and OspC was detected in 10/12 *B. afzelii* and 3/3 *B. bavariensis* while the presence of OspA and OspB was detected in 2/12 *B. afzelii*; all these strains were resistant to NHS. The presence of OspA or OspB was detected in strains susceptible to NHS (1/2 *B. valaisiana* and 8/12 *B. lusitaniae*). The presence of OspA and OspC was observed in a rather heterogenic group of strains, 1/2 *B. valaisiana* was resistant to NHS, while 2/2 *B. garinii* and 3/12 *B. lusitaniae* were susceptible to NHS; 2/3 *B. lusitaniae* from Serbia from this group, were more motile than other *B. lusitaniae* strains and equally motile as a human isolate from Portugal (presence of OspA, OspB, and OspC) which was also susceptible to NHS.

Conclusion
We demonstrated the heterogeneity regarding the presence or absence of proteins within *Borrelia* species. Based on protein profile and serum resistance, we showed that local borrelia strains can have different pathogenic potential and possibly cause LB in humans. Further research of serum-resistant *B. valaisiana* strain is specifically interesting to elucidate the mechanisms of resistance of this species, as well as the potential role of *B. lusitaniae*, the predominant *Borrelia* species in Serbia, in LB epidemiology.
Intravital Microscopy Reveals Novel Motility Defects within Murine Tissues for a Borrelia burgdorferi cheY2 Chemotaxis Mutant

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Introduction
Borrelia burgdorferi (Bb) is an extracellular spirochetal bacterium that causes Lyme disease. Chemotaxis/motility genes play an essential role for the natural enzootic cycle between ticks and vertebrate animals, as these bacteria must sense their environment to rapidly migrate within and between their natural hosts. The response regulator gene(s) (cheY) is important for changing direction within different environments, and Bb is unusual in that it possess 3 different forms of this gene (cheY1-3). This study will assess the role of cheY2 on Bb motility/chemotaxis/virulence.

Methods
Intravital confocal fluorescence microscopy (IVM) will visualize the effects of the cheY2 gene on motility and chemotaxis within murine skin tissues, the assessment of interactions with innate immune cells, and qPCR to determine bacteria levels in target tissues.

Results
Intradermal injection into murine skin showed that the cheY2-deletion mutant (ΔcheY2) reached significantly lower levels in skin tissues than wild-type (WT) Bb, both early and late during infection. ΔcheY2 bacteria were able to migrate to multiple distant tissues, but levels were significantly lower than WT Bb. While WT Bb were able to achieve a constant level and persist long-term, the ΔcheY2 demonstrated much lower numbers, though a subset were able to persist long-term within certain tissues. While WT Bb elicited a strong Bb-specific IgG response, the ΔcheY2 Bb did not elicit significant Bb-IgG, even though bacteria persisted for up to two months. Direct counting of Bb numbers in ear skin demonstrated that ΔcheY2 numbers were similar to WT until Day 5 post-infection and their subsequent peak numbers were >10-fold less than WT until Days 10-21, where the numbers were again similar to WT. While WT numbers remained constant after Day 28, ΔcheY2 numbers gradually dropped to 100-fold less than WT and were cleared in many mice, though some exhibited persistent ΔcheY2 through Day 300 post-infection. WT Bb needed 7-10 days to migrate from one ear to another, but ΔcheY2 Bb required over 60 days to achieve this migration. Direct observation of ΔcheY2 indicated that these mutants reversed their motility direction at a much higher rate than WT, starting at 6h and persisting over 300 days post-infection. Dissemination from the injection site was significantly slower than WT, and these mutants also demonstrated significantly slower average/maximum motility velocities. Direct observation also noted larger numbers of macrophages migrated into ear tissues containing ΔcheY2 compared to WT, though macrophage velocities and distances traveled were similar. Migration of neutrophils into infected tissues and their persistence in ear tissues were similar between ΔcheY2 and WT Bb.

Conclusion
These data demonstrate a chemotaxis/motility gene in Bb that is differentially active depending
on their environment (*in vitro* vs *in vivo*), and provides useful information on aspects of chemotaxis/motility that are important for persistence in mice.
P090 - A point-of-care vertical flow serodiagnostic assay for Lyme disease

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Introduction
Serodiagnostic assays for Lyme disease lack sensitivity in early infection. This is due to a variety of reasons including: nonspecific epitopes in antigens causing high background, the use of single antigen targets, variability in target antigens, and variability of human immune responses. To address these issues, we are creating a multi-peptide, point-of-care (POC) vertical flow serodiagnostic assay (VFA) read by an inexpensive cell-phone based reader and a machine learning diagnostic algorithm that defines positivity based on patterns of antibody binding to multiplexed antigens.

Methods
We mapped the linear B cell epitope of 22 Borrelia burgdorferi (Bb) antigens expressed during mammalian infection. We screened peptides containing the identified epitopes by ELISA, and then in the VFA format using serum sets from Lyme disease patients (early localized, early disseminated, and late disseminated). For the VFA format, peptides were spotted on a nitrocellulose matrix in duplicate in defined spots in a custom 3D printed cassette. The cassette consists of two tops, one of which contains sample buffer for sample loading and introduction onto the peptide loaded sensing membrane, and the other contains gold-nanoparticle-labelled anti-IgM/IgG. Following the procedure, the color produced by immobilization of the detection antibodies is detected using a custom mobile phone reader.

Results
Most antigens contained a mixture of epitopes, some unique to Bb; others ‘cross-reactive,’ binding with antibody in many control serum, healthy and other diseases. We characterized peptides based on their sensitivity and specificity. Some peptides of interest had a higher sensitivity (>50%) with a moderate to high specificity (>90%). Others had a lower sensitivity (<25%) but very high specificity (>98%) and bound sera antibody that were not detected by other peptides. Twenty-eight peptide-epitopes were selected for analysis in the VFA system. The vertical flow format allows for the independent evaluation of antibody binding to individual antigens located in discrete spots. A heat map of signal intensities was generated, and a T-score was calculated for each antigen target. Peptides were ranked by T-score, and a total of nine were selected based on performance in VFA format which were classified in three categories as being sensitive, moderately sensitive, and low sensitive. These peptide targets are currently being used with a set of clinical samples obtained from the Bay Area Foundation Lyme disease Biobank to train a neural network based deep-learning algorithm that will be used to define positivity by a pattern of antibody binding to the nine peptides in the assay.

Conclusion
A highly sensitive, specific rapid POC multiplex assay using a machine learning algorithm to identify patterns of antibody detection would offer laboratory support for Lyme disease diagnosis at the POC, reduce missed diagnoses and improve outcomes through earlier treatment, limiting morbidity associated with disseminated infection.
P091 - First detection of Jingmen tick virus in Corsica: Development and validation of a real time assay to face a potential emergence

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Introduction
The last ten years have witnessed a number of public health burden of emerging infectious diseases. Therefore, investigate the sources of infections to develop surveillance systems and create preparedness and response strategies is crucial for future epidemics. Corsica is a sentinel area for the study of tick-borne diseases. Its geolocation, the presence of avian migration corridors and a warm climate facilitate the emergence of new vector species and associated vector-borne pathogens. Through monitoring vector-borne viral agents on the island with developing rapid diagnostic systems, we can implement preparedness and response of potential epidemics in Europe. A diagnostic system is therefore important to understand the dynamics of an epidemic as a whole through a One Heath approach. Jingmen Tick virus group, new segmented viruses discovered in China, is showing worrying epidemiological results. There are a number of clues to its potential as an emerging arbovirus, including experimental replication and trans-ovarial and trans-stadial passage of the virus in ticks and viremia and virus isolation in humans associated with symptoms.

Aims
The aim of this study was to develop a rapid diagnostic system for the JMTV genome detection and to investigate the circulation of this virus in ticks collected from animals in Corsica.

Methods
A new Real-time PCR system was designed and evaluated for the detection of JMTV group. Ticks collected from domestic and wild animals have been tested with this new diagnostic assay. Complete genome of the virus was sequenced by NGS method. Infection rates were calculated as the maximum-likelihood estimation (MLE) with 95% confidence intervals (CI).

Results
A total of 6,269 ticks collected during 2018-2020 from cattle, horses, wild boars and sheep were grouped into 1,715 pools of 1-6 ticks. Jingmen tick virus DNA was detected in 21 tick pools collected from three cattle and in one tick pool collected from a sheep. The highest JMTV DNA prevalence (MLE=0.58%, 95% CI: 0.35%-0.6%) was observed for Rhipicephalus genus. Hyalomma marginatum and Rhipicephalus bursa collected from the same host were detected positive to the JMTV DNA. Sequencing showed that Corsican JMTV strains were closed to Kosovo JMTV strains.

Conclusion
This study describes the first detection of JMTV in Corsica and more widely in the south-western Mediterranean and allowed to the development of a diagnosis system assay. Future research aimed at defining the origin, the ecology and the spillover potential of JMTV will be critical to understand its relevance to public health.
P092 - Measurement of Antibodies Produced by Antibody-Secreting Cells in Lyme Disease Patients Reveals Three Distinct Types of Humoral Responses

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INTRODUCTION
Measurement of antibodies produced by circulating antibody secreting cells (ASC) has been shown to: 1) detect ongoing infections earlier than serum antibody responses, 2) provide a biomarker for the resolution of a recent infection, 3) enable the diagnosis of recurrent infections among recently infected patients; and 4) identify patients groups unlikely to suffer recurrence. These attributes of ASC have been demonstrated in infections caused by viruses (influenza virus, respiratory syncitial virus), and bacteria including *Staphylococcus aureus*, *Clostridioides difficile*, *Streptococcus pneumoniae*, and *Mycobacterium tuberculosis*.

AIMS
The goal is to assess the utility of ASC-based measurement of pathogen-specific antibodies as a tool for diagnosis and tracking the success of therapy in patients with Lyme disease. A second objective is the description of distinct responses elicited by *Borrelia burgdorferi* infections.

METHODS
Thirty-eight (38) patients with ongoing *B. burgdorferi* infections indicated by erythema migrans (EM) rashes were enrolled at sites in southern Maine and in Baltimore, MD, during the summers of 2018-2020. Whole blood and serum samples were shipped to Atlanta for processing, storage and analysis. Follow-up samples were collected in the first few weeks and then periodically up to one year following initial EM onset. Sera were clarified and stored in frozen aliquots. Whole blood was processed to yield MENSA (Medium Enriched for Newly Synthesized Antibodies), a cell culture-based sample containing only antibodies produced by circulating ASC. Antibodies in serum and MENSA were measured using Luminex immunoassays for seven *B. burgdorferi* proteins and the C6 and C10 peptide antigens.

RESULTS
Patients responded in three distinct ways. Many patients (Canonical responders; n=17) exhibited characteristic, humoral responses to their *B. burgdorferi* infections: rise of anti-C6, anti-C10 or anti-protein IgM and IgG in MENSA and serum in the first weeks following infection, and then exhibited steep declines in MENSA followed by substantial reductions in serum Ig after resolution of the infection. A second group, non-responders (n=10), failed to seroconvert. Non-responders typically had smaller EM rashes (d~5 cm) and short-lived, MENSA responses. The third group (Prolonged responders; n=7) resolved their primary symptoms but continued to produce ASC against C6 and C10 after 6-12 months. The late antibody produced was predominantly IgM.

CONCLUSIONS
Measurement of ASC using MENSA provides a new means for assessment of the human antibody response to infection by *B. burgdorferi*. Here, we provide a first examination using this ASC-based method to measure features of the humoral immune response difficult to measure using conventional serology. Primary diagnosis, resolution of recent infections and distinct classes of humoral responses were observed in this patient population. Further studies will determine
whether the different antibody responses observed by MENS\(\text{A}\) testing correlate with duration of symptoms following treatment.

P093 - Comparison of rapid C6 and CXCL13 assays with CLIA, WB and IDEIA methods in patients suspected of having Lyme borreliosis with neurological symptoms
Introduction

Laboratory diagnosis of Lyme borreliosis (LB) and neuroborreliosis (LNB) is mainly based on detection of *Borrelia Burgdorferi* specific IgM and IgG antibodies in serum and cerebrospinal fluid (CSF). In recent years, chemokine CXCL13 in CSF has been proposed as an additional biomarker of LNB. In this study, the results of routinely used serological methods (chemiluminescence immunoassay [CLIA], western blot [WB], CSF antibody index) were compared with rapid CXCL13 and C6 IgG assays.

Methods

Paired serum and CSF samples from 67 patients suspected of having Lyme disease with neurological symptoms were analysed. All samples were tested for *B. burgdorferi* specific IgM and IgG using CLIA (Liaison; DiaSorin, Italy). Confirmation was made with WB (Virotech, Germany). All reactive first-tier CSF samples were confirmed with IDEIA Lyme Neuroborreliosis (Oxoid, UK) antibody index. Based on the two-tier results and clinical symptoms, the patients were divided into groups of definite LB/LNB and non-LB/LNB. Paired 46 serum and 46 CSF samples from 28 LB/LNB and 18 non-LB/LNB patients were tested with ReaScan+ C6 LYME IgG (Reagena, Finland). Furthermore, 21 and 46 CSFs from LNB and non-LNB patients were tested with ReaScan CXCL13 (Reagena, Finland), respectively. Out of them, 40 CSFs (23 LNB, 17 non-LNB) were tested with both C6 and CXCL13 rapid tests. All assays were performed according to the manufacturer’s instructions.

Results

In the serum sample panel of 46 patients, ReaScan+ C6 LYME IgG showed a comparable sensitivity (92.9%) to CLIA IgG (96.4%) and WB (96.3%). Sensitivity of C6 rapid test in CSF (82.1%) was better than IDEIA IgG (66.7%) but slightly weaker than in CLIA IgG (92.9%). Specificity of rapid C6 test in serum (94.4%) was higher than that of CLIA IgG (77.8%). In CSF, the specificity of rapid C6 test and CLIA IgG was 88.9% and 94.4%, respectively. WB IgG and IDEIA Neuroborreliosis IgG showed negative results for all non-LB/LNB samples. The amount of false positive results in CLIA IgM was high showing specificity of 38.9% for CSF and 55.6% in serum. ReaScan CXCL13 showed high specificity (97.9%) in 46 non-LNB CSF samples. Sensitivity of CXCL13 rapid test was moderate, 52.4% and 66.7% with the cut-off 500 pg/ml and 250 pg/ml, respectively. Comparison of rapid C6
and CXCL13 tests in 40 CSFs showed comparable specificity (94.1%), but rapid C6 test showed higher sensitivity (82.6% vs. 34.8%).

**Conclusion**

Routine diagnosis of LNB should be prompt and reliable for successful treatment. Rapid ReaScan+ C6 LYME IgG and ReaScan CXCL13 tests are good additional tools that can improve, simplify, and speed up LNB diagnosis. CXCL13 must be analysed using fresh samples since storing of the samples in a refrigerator or freezer may have an impact on the results. However, this was not seen in the C6 assay.
P094 - Diagnosis and prevalence studies of babesiosis, an emerging zoonotic disease in Scotland

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Introduction
Human babesiosis in Europe is a rare disease with about 60 cases reported since the first diagnosed case in 1956. Most European cases have been attributed to Babesia divergens or Babesia microti, with fewer but fatal cases due to Babesia venatorum. Additionally, a case of Babesia crossa-like, previously unknown in Europe, was reported last year, along with a case attributed to the newly identified species, Babesia divergens-like FR1.
Globally, the detection of human cases of babesiosis has increased following the expansion of serological testing, and the number of cases is predicted to rise due to climate change. The prevalence of Babesia infection has been reported to vary depending on the area, and population tested. Studies of Babesia in humans in the UK have been limited by the number of samples, as diagnostic testing is only undertaken in suspected symptomatic individuals. Serological testing is performed using the Indirect Fluorescent Antibody Test (IFAT), but, until recently, no Babesia divergens slides were commercially available. The IFAT technique is notoriously time-consuming, and interpretation of results can be challenging, depending on the competence and experience of the technician. Thus, a test that can be routinely performed is required to diagnose human babesiosis.

Aims
The present study aims to develop a novel Enzyme-Linked Immunosorbent Assay (ELISA) for European Babesia spp. This could facilitate an increase in sample numbers being tested and would assist in estimating parasite prevalence among humans that have experienced tick bites.

Methods
We are undertaking a systematic approach to identifying ELISA candidate antigens representing European zoonotic Babesia spp. Top-ranking antigen-encoding genes are being cloned into bacterial expression vectors, recombinant proteins expressed, and their immunoreactivity investigated using a panel of Babesia-positive serum samples from both humans and animals. A subset of promising antigens will be selected and developed to cover all zoonotic Babesia spp. Validation of ELISA antigens will include cross-reactivity testing against other common protozoa genera known to infect humans.

Results
Based on the literature review, Babesia divergens antigens AMA1, 37kDa, RAP1, and EBP have been identified as promising candidates. Two other antigens, BdRON2 and BdHSP70, have been excluded based on a lack of evidence of immunogenicity in infected hosts and a high level of
sequence conservation among species. *Babesia microti* proteins BmSA1 and BMN1-17 will also be analysed among the other published antigens. The results of gene cloning and the expression of recombinant antigens will be presented.

**Conclusion**
An ELISA test for zoonotic *Babesia* spp. will improve diagnosis and more accurately assess the emergence of human babesiosis in Scotland and elsewhere in Europe. A more detailed assessment of the prevalence of this zoonotic pathogen and its potential to exacerbate disease syndromes caused by other tick-borne pathogens of humans is required.

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Introduction
Serologic assays (enzyme immunoassay [EIA] and immunoblots [IB]) for detection of Borrelia burgdorferi (BB) and nucleic acid amplification tests (NATs) for detection of Anaplasma phagocytophilum (AP), Ehrlichia spp. (EHR) and Babesia microti (BM) are commonly available diagnostic assays. Here we present tick-borne disease (TBD) prevalence and test utilization patterns over 8 years for our rural healthcare system in the Upper Midwest USA.

Methods
Laboratory services for our 10 hospital and 55 community clinic system in the northern half of Wisconsin State are delivered in a hub-and-spoke model. Interrogation of the laboratory information system based on TBD test order codes was performed for the years 2013-2020 and included serologic tests for BB (CDC standard two-tier test [STTT] algorithm), and NATs for AP, EHR and BM. Total assays performed and percent positivity were summarized by month/quarter/year, excluding temporally-related test duplicates.

Results
7.1% of 57,851 patient sera tested positive for BB over the 8-year period (range by year 4.4-9.3%) using the STTT algorithm. While 14.0% of the screening EIA tests were reactive (11.7% positive and 2.3% equivocal), IB testing confirmed positivity in only half with 1.5% IgG, 3.5% IgM or 2.1% for both antibody tests. Total NATs performed (% positive; range over 8 years) for AP, BM and EHR included 26,647 (2.29%; 1.16-3.42%), 26,306 (0.46%; 0.07-0.73%) and 26,648 (0.13%; 0.07-0.25%), respectively. Coinfections detected simultaneously included 125 BB/AP, 42 BB/BM, 5 BB/EHR, and 2 BB/BM/AP. Of all patients with AP 20.8% were also seropositive for BB. AP and EHR were not detected in the first quarter of any year despite 1,981 tests being ordered. Yearly BB prevalence remained relatively constant whereas prevalence of AP and BM was more variable. EHR species identified included 21 E. muris subsp. eauclarenensis and 1 each E. chaffeensis and E. wingii/E. canis.

Conclusion
BB continues to be the primary TBD pathogen diagnosed in our region for all years studied with 7.1% of specimens STTT positive followed by 2.29%, 0.46% and 0.13% positive for AP, BM and EHR, respectively, using NATs. Coinfections remain rare with most consisting of BB/AP and secondarily BB/BM. The prevalence of BB is likely higher given many experienced providers in the endemic zone treat clinically typical cases empirically and without laboratory support.
Introduction
Lyme Disease (LD) is an increasing public health issue in the UK, especially in rural regions. This may be due to changes in the environment and in human behaviour. The ability to accurately recognise Lyme cases and record associated data at a primary care level needs strengthening to improve patient management and disease reporting.

Aims
The aim of the project is to:
- Standardise diagnosis, management and treatment of Lyme Disease and improve coding in order to enable collection of accurate incidence data in a UK General Practice sample.

Methods
This Pfizer-funded educational project is a collaboration between NHS Highland and Pfizer Inc, with partners Albasoft Ltd and Scotland’s Rural College. This Service Evaluation project is testing a new model of delivering support for diagnosis, management and treatment of LD within general practice. It also gathers data to better define the local epidemiology of LD.

An interactive tool (IT) was developed and placed on the GP database in 15 NHS Highland GP Practices to support the capture of relevant epidemiological data during patient visits (following a tick bite or for potential LD). Additionally, a comprehensive training package for GPs was deployed to support LD awareness, education, diagnosis and management. Data were collected on all patients visiting those Practices between September and December 2021. Cameras were provided for Practices to take educational illustrative clinical photographs.

A survey questionnaire of participating Practices was conducted to measure satisfaction.

Results
The initial data has been analysed and has revealed some interesting results:
- 69 patients were included in the sample
- Participating GPs were very satisfied with the IT model, the training and educational support they received, and most would seek to use this approach with all patients presenting with possible LD
- Comparison with incidence data from the same GP practices from the same time period in previous years (2019 = 4.5, 2020 = 7.4 and 2021 = 9.4 per 10,000 GP population) suggests this intervention can result in improved case ascertainment at a primary care level which will lead to the availability of more robust incidence estimates
- A range of factors appear associated with the likelihood of acquiring LD
- Relationships between factors suggests further ways to support GPs in managing LD

Conclusions
This pilot project has raised issues that will be followed up in the next stage, which will include 15 additional GP Practices in Scotland and 10 in England (UK 40) and will run until the end of
2022. If the project shows improved LD diagnosis and management, the tool could be offered to GPs across the UK.
The development and validation of a protein array for the detection of antibodies against the tick-borne pathogen *Borrelia miyamotoi*

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**Introduction**

Current serological tests for the emerging tick-borne pathogen *Borrelia miyamotoi* lack diagnostic accuracy. To improve serodiagnosis, we investigated a protein array simultaneously screening for IgM and IgG reactivity against multiple recombinant *B. miyamotoi* antigens.

**Materials and methods**

The array included six *B. miyamotoi* antigens: glycerophosphodiester phosphodiesterase (GlpQ), multiple variable major proteins (Vmps) and flagellin. Sera included samples from PCR-proven *Borrelia miyamotoi* disease (BMD) cases, multiple potentially cross-reactive control groups (including patients with culture-proven Lyme borreliosis, confirmed Epstein-Barr virus, cytomegalovirus or other spirochaetal infections), and several endemic and non-endemic healthy control groups. Based on ROC-analyses, the cut-off for reactivity per antigen was set at 5 μg/ml for IgM and IgG.

**Results**

The individual antigens demonstrated high sensitivity, but low specificity for both IgM and IgG. The best-performing single antigen (GlpQ) showed a sensitivity of 88.0 (95%CI 78.9-93.5) and a specificity of 94.2 (95%CI 92.7-95.6) for IgM/IgG. Applying the previous published diagnostic algorithm - defining seroreactivity as reactivity against GlpQ and any Vmp - revealed a significantly higher specificity of 98.5 (95%CI 97.6-99.2), however a significant lower sensitivity of 79.5% (95%CI 69.3-87.0) for IgM/IgG, as compared to GlpQ alone. Therefore, we propose to define seroreactivity as reactivity against GlpQ and any Vmp or flagellin, which resulted in a comparable sensitivity of 84.3% (95%CI 74.7-90.8), and a significantly higher specificity of 97.9% (95%CI 96.9-98.7) for IgM/IgG, as compared to GlpQ alone.

**Discussion/conclusions**

We have developed a novel serological tool to diagnose BMD that could be implemented in daily clinical practice and used in epidemiological studies.
P098 - Improving the Diagnostic Value for Serology: Results from 12 FDA-Cleared Assays on Lyme Disease Biobank (LDB) Samples Collected Between 2017-2020

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Introduction
LDB collects samples from individuals with signs and symptoms of early/acute Lyme disease (LD) and endemic controls to facilitate research in LD diagnostics.

Methods
Samples collected from East Hampton, NY, and Marshfield Clinic, WI, from 2017-2020 were tested in a blinded fashion at Mayo Clinic (MC) and Stony Brook University (SB) as described.[Horn 2020] MC testing included PCR for Borrelia burgdorferi and serology using the standard two-tiered testing algorithm (STTTA) (i.e. VlsE/pepC10 ELISA (Zeus Scientific) followed by IgM and IgG immunoblots (Viramed)). C6 peptide ELISA (Oxford Immunotec) testing was performed at SB, however C6 was discontinued in late 2019.

Serum samples from 251 first draws (107 cases and 144 controls) and 69 second draws of cases were interrogated with the first-tier VlsE-OspC ELISA followed by 3 second-tier ELISAs (IgM, IgG, and IgG+IgM) and 2 immunoblots (IgM and IgG) from Gold Standard Diagnostics (GSD). STTTA and modified two-tiered testing algorithm (MTTTA) were applied. Cases (45) categorized as laboratory confirmed (LC) and controls were also tested with Zeus Scientific’s (ZEUS) IgG and IgM second-tier ELISAs, and MTTTA was applied. Blinded testing was performed at GSD, with second-tier tests run regardless of first-tier results.

Results
42 cases were categorized as LC using serology results from the first draw. Of the 31 samples STTTA-positive at MC, 30 were positive by additional STTTA or MTTTA; 9 other LC samples were MTTTA-positive. For those samples testing positive by STTTA or MTTTA, 90% were positive by multiple algorithms. MTTTA performed better than STTTA in early LD (37 GSD MTTTA; 36 ZEUS MTTTA; 24 GSD STTTA; 31 MC STTTA). All 144 controls were STTTA-negative, and 4 were MTTTA-positive (GSD 1; ZEUS 3).

Of the 62 cases not categorized as LC by LDB, 18 (29%) were positive on a single tier, 1 was MTTTA-positive (2%), and 43 (69%) were negative on all FDA-cleared serological tests performed. Of these 62 cases, 58 (94%) were enrolled with a provider-diagnosed EM >5 cm, a clinical diagnosis of LD. IgG seroconversion (defined as positive IgG blot on second draw when negative on first draw) occurred in 2 samples. Of the 107 cases in this analysis, 97% had antibiotics prescribed at the initial visit, suggesting seroconversion may be rare after treatment for early LD.

Conclusion
Additional knowledge about a sample cohort is gained when samples are tested with multiple FDA-cleared assays. MTTTA identified more early LD samples than STTTA. Patients with early LD whose samples are positive by STTTA or MTTTA test positive on multiple assays. However, there is a subset of patients with a clinical diagnosis that test negative with current testing algorithms,
and novel diagnostics are needed.

P099 - Self-Administered Computerized Neuropsychological Assessment in Post-Treatment Lyme Disease Syndrome

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Introduction
Previous studies from our center and others have identified a pattern of neuropsychological deficits in patients with Post-Treatment Lyme Disease Syndrome (PTLDS). These most commonly include problems with processing speed, memory, and language abilities. Neuropsychological assessments, however, may not be available outside of urban areas or more specialized hospital settings. Self-administered computerized neurocognitive testing may be implemented to provide this service to a broader segment of the population, but its sensitivity to deficits in PTLDS is unknown.

Aim
Assess the feasibility and sensitivity of a self-administered computerized test battery to detect neurocognitive deficits in PTLDS.

Methods
58 patients who met criteria for either Definite (n=41) or Probable (n=17) PTLDS were compared to 25 Healthy Volunteers on a battery of tasks from the Neurotrax™ computerized assessment battery. Tasks were administered by computer with instructions provided on screen by the program itself, with little supervision required by clinic staff. The battery used in this study included ten tasks that produced standardized composite scores (based on internal normative data) in 7 domains of functioning: Motor Skills, Information Processing Speed, Attention, Memory, Verbal Functioning, Visual-Spatial Functioning, and Executive Functioning. Groups were compared across domains in an omnibus analysis via the General Linear Model, with Greenhouse-Geiser correction, followed by univariate comparisons.

Results
In a preliminary analysis, Definite and Probable PTLDS patients did not differ from one another in any domain, on a composite average of all domains, nor on any one of 23 subscores within domains, and were therefore pooled for comparison to Healthy Volunteers. In comparison to Healthy Volunteers, this pooled PTLDS patient group did not differ in aggregate test performance (Group Main Effect, F[1364.4,5.7]=3.77, p =.067), but did differ in their performance across the domains (Interaction Effect, F[665.5,2.8]=3.77, p =.034). In comparisons of individual neuropsychological domains, PTLDS patients performed significantly more poorly on Information Processing Speed only (F[1,57]=9.43, p=.003). Group differences in the Attention (F[1,57]=2.98, p=.088), Memory (F[1,57]=3.16, p=.079), and Executive Functioning (F[1,57]=3.63, p=.060) domains approached, but did not reach statistical significance.

Conclusions
Self-administered, computerized testing produced a pattern of performance in PTLDS patients that approximated that found in studies using examiner-administered measures, but with less robust differences between groups - with the exception of Information Processing Speed.
measures. In prior neuropsychological studies, memory deficits - usually assessed via recall tasks administered by an examiner - are typically the most prominent deficits in PTLDS samples but were less distinct using the recognition task in this self-administered battery. Self-administered computerized tasks provide an approximation of deficits observed with standard examiner-administered tasks but may be less sensitive. These assessments may be useful, however, if standard examiner-administered assessment is not available.
P100 - Evaluation of the new Borrelia All-In-One ViraChip® protein microarray for the serological diagnosis of Lyme Borreliosis in a prospective study.

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Introduction

Serologic testing for detection of antibodies against members of the Borrelia burgdorferi sensu lato complex begins with a first-tier screening test, followed by confirmatory testing of initially reactive samples using an alternative assay(s). The standard two-tier testing algorithm relies on an initial screening enzyme immunoassay (EIA) followed by IgM and IgG immunoblots for confirmation of initial borderline or positive results. More recently, a modified two-tier testing algorithm has been endorsed, which relies on one or multiple EIAs as the second tier assays.

Methods

A microarray antibody detection system, the Borrelia All-In-One ViraChip® (Viramed Biotech AG, Germany) was recently developed, which combines the two-tiered testing approach in a single well of a microtiter plate. The microarray has 11 B. burgdorferi antigens adhered to a nitrocellulose membrane in the microtiter well, among which reactivity at the VlsE antigen is used as the first-tier screen. If antibody reactivity is observed at the VlsE antigen, reactivity at the remaining 10 antigens is assessed. Reactivity by the All-In-One assay is determined by measuring the optical density at each antigen using the ViraChip® Reader as an instrument. Ultimately, the All-In-One assay allows for streamlined performance of two-tier in a single microarray well.

Aims

To evaluate performance of the All-In-One assay, we conducted a prospective study consisting of 1500 serum specimens submitted between January 20 and February 10, 2022 to Mayo Clinic Laboratories (Rochester, MN) for routine Lyme disease serologic testing using either the standard or modified two-tiered testing algorithm. All samples were tested by the All-In-One assay and by both the standard and modified testing algorithms.
Results

The All-In-One assays showed an overall agreement of 93.9% and 94.1% with the modified and standard two-tiered testing algorithms, respectively.

Conclusion

This study indicates that the All-In-One B. burgdorferi protein microarray approach for serologic testing for diagnosis of Lyme disease is a promising new diagnostic tool which performs similar to currently available methods. The primary benefit of this assay is the ability to perform high-throughput, automated, multi-tier testing for Lyme disease in a single microtiter well, which incorporates a large number of B. burgdorferi antigens, with minimal technologist hands-on-time.
**P101 - Performance of the new Borrelia All-In-One ViraChip® protein microarray for the serological diagnosis of Lyme Borreliosis in a retrospective study.**

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**Introduction**

Serology for detecting antibodies against *Borrelia burgdorferi* s.l. normally consists of a first test for screening and a second test for confirmation. The Standard two tier (STT) protocol may use an Enzyme-Immunoassay (EIA) for screening followed by an immunoblot test for confirmation of borderline or positive results from the screening test. Two test approaches employing an EIA as the first test and one or multiple EIAs as the second test were evaluated over the last years and may be used as modified two tiered (MTT) protocols alternatively. A microarray consisting of multiple Borrelia antigens, the Borrelia All-In-One ViraChip® combines a two test approach and a multiplex antibody detection system in one well of a microtiter plate. VlsE is being used for the screening test and, when reactive, is followed by the analysis of multiplex antigens for confirmation. Thus, two independent test results can be obtained from one microarray streamlining the two test approach to one step all in one well.

**Methods**

In a retrospective study consisting of 280 well-characterized serum specimens from the CDC premarket panel the performance of the Borrelia All-In-One ViraChip® was compared to the standard two tier protocol and a modified two tiered testing protocol.

**Results**

The results show that the Borrelia All-In-One ViraChip® displayed sensitivities of 90 % (Lyme Disease, Stage I), 100 % (Lyme Disease, Stage II) and 100 % (Lyme Disease, Stage III). Specificity in the panel of Healthy Controls was 99 % and in the panel of Disease Controls 98 %. Results for the STT / MTT yielded sensitivities of 73 % / 78% (Lyme Disease, Stage I), 90 % / 100 % (Lyme Disease, Stage II) and 100 % / 100% (Lyme Disease, Stage III) whereas specificities were determined as 100 % / 99 % in the panel of Healthy Controls and 99 % / 97 % in the panel of Disease Controls.
Conclusion

This study shows that the Borrelia All-In-One ViraChip® protein microarray is an efficient and promising new diagnostic tool for Lyme Borreliosis in routine testing. With high sensitivities and specificities in combination with a high degree of automation, stable, reproducible, and detailed results can be obtained with little hands-on-time in one step analysis. Furthermore, by using the Borrelia All-In-One ViraChip® technology antigen reactivity patterns may give indications about the stage of Lyme Borreliosis.
**P102 - The clinical value of CXCL13 in suspected Lyme neuroborreliosis: a retrospective case-control study**

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**Introduction**
Currently available tools to confirm the diagnosis of definite Lyme neuroborreliosis (LNB) has limitations. Intrathecal production of antibodies against *Borrelia burgdorferi* may be absent in early stage of the disease and persist long time after successful antibiotic treatment. It has been demonstrated that chemokine CXCL13 is highly elevated in the cerebrospinal fluid (CSF) during early LNB and decreases rapidly after antibiotic treatment. Our aim was to evaluate the clinical relevance of this marker in the diagnosis of LNB.

**Methods**
We conducted a retrospective case-control study including all patients that underwent CXCL13 examination of the CSF at the Department of Infectious diseases, University hospital Bratislava, Slovakia, between 1 September 2019 and 30 April 2022. The patients were classified using the European Federation of Neurological Societies (EFNS) guidelines on the diagnosis and management of European LNB as cases with definite or possible LNB or as controls with no LNB. ReaScan CXCL13 lateral flow immunoassay (LFA) rapid test was used on fresh samples at the time of diagnosis and Euroimmun CXCL13 enzyme-linked immunosorbent assay (ELISA) was used on frozen samples in May 2022 (results pending). We examined sensitivity and specificity of LFA test and will evaluate the correlation between CXCL13 levels obtained by LFA CXCL13 and ELISA CXCL13 test.

**Results**
Of 139 patients, 53% were women and 49 (36%) were children < 18 years of age. 32 patients were classified as definite LNB, 5 as possible LNB and 101 as non-LNB controls. CXCL13 LFA yielded positive result in 28 patients with definite LNB, 2 with possible LNB and 2 controls (1 neurosyphilis and 1 tick-born encephalitis). Comparing patients with definite LNB with controls, sensitivity and specificity of LFA was estimated at 81.2% and 98.0% respectively (corresponding cutoff value of positive result, 250 pg/ml; area under the receiver operating characteristic [ROC] curve, 0.90). After excluding from analysis 29 patients taking antibiotics prior CFS examination, sensitivity and specificity was 92.3% and 97.5% respectively.

**Conclusion**
LFA CXCL13 rapid test is a suitable additional diagnostic tool of patients with suspected LNB. Its use shortens the diagnostic process and may reduce unnecessary antibiotic prescription. Antibiotic treatment before CSF examination reduces the diagnostic value of the test.
P103 - Development of novel immunoassays for the serodiagnosis of louse-borne relapsing fever

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Introduction
Louse-borne relapsing fever (LBRF) caused by Borrelia recurrentis is a poverty-related and neglected infectious disease with an endemic focus in the Horn of Africa. Re-emergence of the disease occurred in Europe during the refugee crisis in 2015 and sporadic outbreaks were frequently reported in eastern Africa. Currently, there are no validated immunoassays available for the serodiagnosis of LBRF.

Aims
The aim of this study was to develop novel and reliable immunoassays by investigating clinically suspected and culture-confirmed serum samples from LBRF patients as well as a broad panel of control serum samples from patients with other spirochetal, bacterial, and parasitic diseases.

Methods
Two in vitro immunoassays for the serodiagnosis of louse-borne relapsing fever have been developed by employing a line blot and an ELISA-based system to analyze the IgM and IgG immunoreactivity of patient and control sera.

Results
We identified two immunoreactive antigens (the complement-inhibiting protein CihC and the glycerophosphodiester phosphodiesterase GlpQ of B. recurrentis) as the most promising target candidates leading to the evaluation of two prototypic immunoassays (line blot immunoblot and ELISA) for IgM and IgG. To optimize the IgM immunoassay which revealed a lower sensitivity for CihC, we conducted a bioinformatic approach to localize the relevant immunogenic regions within this particular protein. By utilizing a N-terminal CihC fragment, the sensitivity and specificity of both immunoassays (CihC and GlpQ) were significantly improved (IgM: sensitivity 100%, specificity 89.9%, IgG: sensitivity 100%, specificity 99.2%).

Conclusion
In conclusion, our findings indicate the diagnostic potential of CihC and GlpQ as valuable markers for the serodiagnosis of LBRF even at early time points of infection. Moreover, the data collected herein provide strong evidence for the utilization of these immunoassays as reliable tools in clinical practice.
P104 - No correlation between symptom duration and intrathecal production of IgM and/or IgG antibodies in Lyme neuroborreliosis - a retrospective cohort study in Denmark

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Introduction
In Europe, the diagnosis of definite Lyme neuroborreliosis (LNB) require intrathecally produced *Borrelia*-specific IgM and/or IgG antibodies.

Aims
We aimed to examine if the time from symptom debut to lumbar puncture (LP) correlated with findings of intrathecal production of *Borrelia*-specific IgM and/or IgG antibodies in LNB.

Methods
We conducted a retrospective study of 544 patients with a positive *Borrelia burgdorferi* antibody index (Bb-Al), analysed at the Department of Clinical Microbiology at Odense University Hospital, Denmark, between 01.01.1995-31.12.2020.

Results
The delay from symptom onset to LP was for patients with positive Bb-Al IgM 30 days (IQR 14-95 days), for patients with positive IgG 24 days (IQR 11-62), and 24 days (IQR 14-48) for patients with both positive IgM and IgG, P=0.098. Ninety-three patients had a second, and 25 had a third LP performed after a median of 125 (IQR 28-432) and 282 days (IQR 64-539), respectively, where the majority of patients (66.7%) did not convert from their initial intrathecal antibody finding. The prevalence of different clinical manifestations differed significantly between the three Bb-Al groups.

Conclusion
Intrathecal *Borrelia*-specific antibody production does not follow the otherwise well-known immune response with an initial IgM production followed by an IgG production. Physicians diagnosing LNB patients should not base their interpretation of the stage of the disease on the type of antibodies found in the cerebrospinal fluid.
P105 - Diagnostics of Borrelia burgdorferi sensu lato with multi-locus sequence typing (MLST) –which strains cause infection in humans in Southern Sweden?

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Introduction
Lyme borreliosis (LB) is the most common tick-transmitted disease in Europe causing clinical manifestations in the skin, the central nervous system, and the joints. Diagnosis of LB is based on the patient’s medical history, clinical signs and self-reported symptoms together with the detection of Borrelia-specific antibodies. However, in some cases, PCR can be used as a complement, and currently, real-time PCR is the most commonly used method in clinical laboratories in Sweden. To distinguish between different Borrelia strains multi-locus sequence typing (MLST), a method with the ability to differentiate strains may be used as a supplement to real-time PCR. By determining which strains that cause human infection and how they are correlated to the clinical picture and disease course, treatment recommendations may evolve over time and be more individualised since some strains are more prone to cause severe inflammation. Studies involving typing of Borrelia strains have both diagnostic, clinical, epidemiological, and evolutionary relevance for research on bacterial pathogens.

Aims
The objective of the study is to identify which B. burgdorferi sensu lato strains that cause human infection in the Southern Swedish population and to determine the potential relationship between clinical manifestations and different strains.

Methods
Patients investigated for suspected LB at the National Reference Laboratory for Borreliosis at the Department of Clinical Microbiology, Ryhov County Hospital by real-time analysis during 2019-2022 were included in the study. The samples (cerebrospinal fluid, skin biopsies, and synovial fluid) with positive outcomes in the real-time PCR analysis were further analysed with MLST. Samples were collected from both children and adults.

Results
In total, 54 patient samples have been analysed with MLST at the time of writing. However, eight samples were excluded due to lack of sequence results. In total, 39 samples were determined as B. afzelii, three samples as B. garinii, and four samples as B. burgdorferi sensu stricto. Most of the samples were from skin biopsies (n=32), followed by synovial fluid (n=13), and cerebrospinal fluid (n=1). Among the biopsies, 31 of 32 were determined as B. afzelii and one as B. garinii. The synovial fluid samples contained primarily B. afzelii (n=7), followed by B. burgdorferi s.s. (n=4), and B. garinii (n=2). Most of the patients (n=25) had sought medical care for suspected acrodermatitis chronica atrophicans.

Conclusion
Currently, evaluation of MLST results is in progress and no certain conclusions regarding MLST profiles and the relationship between strains can be made so far. However, the method seems to work well, and with larger study material, we expect to find answers to our aims. In the long term, the study also intends to study the current frequency of different strains and potential epidemiological changes over time.
P106 - Persistence of IgM in Lyme-Borrelia serology.

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Introduction
In commercial immunoassays detection of IgM is usually based on the reactivity to the outer surface protein C (OspC) of Borrelia burgdorferi sensu lato (sl). Persistence of IgM is frequent in Lyme-Borrelia serology and it often occurs in subjects without clinical symptoms of Lyme borreliosis.

Objections/Aims
The aim of the study was to investigate the etiology of persistent IgM and to analyze if there is an association with nonspecific symptoms.

Methods
The study group comprised individuals with persistent IgM antibodies in the absence of IgG. We assessed the differences in immunoblot band intensities between the study group and patients with erythema migrans (EM). We investigated the relation between ELISA values and time elapsed since past EM. Nonspecific symptoms were documented using a questionnaire. The number of symptoms and their improvement were analyzed statistically. In the experimental part of the study, we examined by immunoblotting the reactivity of persistent IgM and the known C-terminal epitope of OspC comprising the aminoacids PKKP. For this purpose we generated variants of OspC with the modified C-terminal epitope. Finally, we carried out a BLAST search in order to identify proteins carrying the same epitope. Two of them - the human tryptase 1 (TPSAB1) and P. aeruginosa magnesium chelatase (Mg Che) - we expressed recombinantly in order to study cross-reactivity with the persistent Borrelia IgM.

Results
Fifty-nine patients (47 women; 80%) were included in the study group. The mean IgM-ELISA values did not change significantly during follow-up (median 6.2 months). We found a similar reactivity to Borrelia antigens in the group with persistent IgM compared to EM patients. Nonspecific symptoms improved significantly more often in individuals with lower IgM ELISA values, but these values did not influence the numbers of nonspecific symptoms. The mean ELISA value in the study group was dependent on time elapsed since past EM. Our findings confirmed that the persistent IgM antibodies were specific for the C-terminal PKKP motif of OspC. Finally we demonstrated that sera of persons with persistent IgM reacted with C-terminal PKKP antigens from both human (TSAB1) and prokaryotic (Mg Che) origins but this reactivity was lost with variants missing the C-terminal PKKP tetramer.

Conclusions
We demonstrate that the C-terminal PKKP motif plays a main role for the reactivity of persistent Borrelia-IgM towards OspC. However, cross-reactivity to other eukaryotic and/or prokaryotic antigens may hamper the specificity of OspC in the serological diagnosis of Lyme borreliosis. This knowledge is essential for the improvement of serologic assays for Lyme borreliosis and provides a cross-reactive explanation for the persistence of Borrelia-IgM.
P107 - Northtick evaluation of IgM testing

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**Introduction**

Serological testing is recommended for all suspected cases of Lyme borreliosis other than those with clinically identified Erythema Migrans rash. Methods to detect IgG antibodies to *Borrelia burgdorferi* lack sensitivity during early disease, and their persistence in serum can complicate interpretation. IgM antibodies to *B. burgdorferi* are produced earlier than IgG, thus should enable the detection of more cases of early Lyme borreliosis than IgG alone and potentially prevent progression to disseminated or late disease. However, IgM tests may have sub optimal specificity and IgM antibodies can also persist.

**Objections/Aims**

To evaluate the benefits, limitations and costs associated with borrelia IgM testing in Scotland and Sweden as part of Northtick, an Interreg North Sea Region project, co-funded by the European Union.

**Methods**

Testing and clinical data for sera tested at the Scottish Lyme Disease and Tick-borne infections reference laboratory (SLDTRL) from 2018 to 2020 was analysed as well as data from sera and CSF tested at the Swedish reference laboratories at Region Jönköpings län (RJL) and Sahlgrenska University Hospital (SUH) during 2017 and 2017-18, respectively.

**Results**

Analysis of Swedish reference laboratory data (RJL) from 2017 (n=4428) observed an estimated false positive rate of 50% for IgM EIA on sera within a single-tier testing protocol. Scottish reference laboratory data from 2018 to 2020 (n=18,189) showed an estimated false positive rate of 25.5% for IgM immunoblot within a 2-tier testing protocol, but 80.1% for IgM EIA. For 2017 and 2017-18, 3/579 (0.5%) and 48/509 (9.4%) of patients with suspected Lyme neuroborreliosis from RJL and SUH, respectively, had raised IgM CSF/serum antibody index only.

**Conclusions**

IgM immunoblot showed clear cost benefits in Scotland as the relatively small increase in laboratory costs were offset by at least 51% savings in societal costs from not missing cases of early Lyme borreliosis that may develop into more serious disease if left untreated. The benefits of IgM EIA in sera were less clear. However, the detection of borrelia-specific IgM antibodies in CSF by EIA and calculation of serum/CSF antibody index may be important for the diagnosis of early Lyme neuroborreliosis (LNB).
P108 - What we learned from testing the ticks – the use of novel testing approaches uncovered unexpected pathogen prevalence in tested ticks

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Background
The high failure rate of tick-borne infection (TBI) testing and treatment underscores the necessity to reassess the pathogen prevalence in infected ticks in order to refine the patients’ testing strategies.

Aims
Following an initial small study on 30 ticks we aimed to conduct a large study (110 tested ticks) comparing different testing approaches for tick-borne pathogens. The goal of this contribution is to bring the focus on the importance to enlarge TBI-related testing targets and shed some light on high prevalence of Borrelia Relapsing fever (RF) strains both in ticks and late stage undiagnosed patients.

Methods
We used Phage-based real-time PCR test for Borrelia (searching for 3 major Borrelia groups (Borreli burgdorferi sl, Borrelia miyamotoi and RF group) and Rickettsia, Hybrispot dot-blot multiplex test for 7 TBIs, and various PCRs for Borrelia burgdorferi sl, Bartonella and Babesia testing.

Results
110 analyzed ticks by Phage test showed 30% positive for B. miyamotoi, 42% for RF (mainly B.hermsii group), 14% positive for both B.miyamotoi and RF and only 10% positive for B. burgdorferi sl. In comparison, Borrelia IVD qPCR detected only 1,8% of B. burgdorferi positive ticks and multiplex Hybrispot revealed 10% positives. Thus, none of 3 different methods used evidenced more than 10% of ticks positive for B. burgdorferi sl, validating thus the results obtained by the Phage test and emphasizing the need to focus on RF strains. The positivity rate for other TBIs were: Rickettsia 10%, Babesia 3,6%, Ehrlichia 1,8%, Bartonella 9%, Anaplasma 2,7%. Notably, 32,7% of tested ticks were positive for more than one pathogen. In addition, over 6000 results from patients (mainly late stage / chronic) are showing over 60 % of tested samples positive for RF strains (mainly B.miyamotoi and B.hermsii).

Conclusion
This is the first large-scale report on prevalence of Borrelia RF strains in ticks, along with an important percentage of Rickettsia positive ticks and over 32% of tested ticks carrying more than one pathogen. Seen this high prevalence of Borrelia RF in tested ticks, further supported by similar high percentages found in patients, the overall high expansion of undiagnosed TBD cases worldwide might be linked to the screening choice focusing only on B. burgdorferi sl and only rarely testing for RF while the later ones seem to be much more prevalent. Enlarged testing of ticks removed by patients could prevent the onset of chronic disease in bitten patients.
Introduction

Borreliosis, Rickettsiosis and Ehrlichiosis are tick-transmitted diseases that cause the so-called emerging and re-emerging diseases of public health importance. Most cases are acquired by the bite of ticks that are infected by the microorganisms. Various mammals contribute to perpetuating the infection and maintaining the biological cycle, but it is pets and farm animals, which are in close contact with humans, that play a very important role in the transmission of these diseases. The lack of specific detection methods and the lack of information on the presence of these diseases limits their prevention and control. The municipality of Jalpan de Serra, in the state of Querétaro, has the species of ticks that transmit these diseases and the climatological factors for the establishment of these vectors, in addition to the fact that in this municipality there is a great mobilization of people and animals between areas where these diseases have been previously reported.

Aims (optional)

The aim of this study was to investigate the presence of antibodies against *Borrelia burgdorferi*, *Rickettsia rickettsii* and *Ehrlichia canis* in dogs from Jalpan de Serra in Queretaro, Mexico.

Methods

A total of 107 dogs (59 males and 48 females) over two months of age, with the presence of ticks in the adult stage, were sampled. Blood samples were collected from each animal in vacutainer tubes without anticoagulant, which were kept at 4°C until processing. The tubes were centrifuged to obtain serum, which was frozen at -20°C until use. Eighty-five sera were tested for antibodies anti-*Ehrlichia canis*, 84 sera were tested for antibodies anti-*Borrelia burgdorferi*, and 79 sera were tested for the presence of antibodies anti-*Rickettsia rickettsii* antibodies. The sera were evaluated by commercial indirect immunofluorescence antibody tests (IFAT) using a goat anti-dog IgG antibody linked to Alexa 488 as a secondary antibody. Commercial sera from dogs infected with each of the pathogens were used as positive controls, and commercial sera from uninfected dogs were used as negative controls.

Results

The results of this study indicated that 50% of the canids presented at least 2 genera of ixodids: *Rhipicephalus* and *Amblyomma*. It is important to note that 46/85 sera were positive for *E. canis* (54.11%), 50/84 sera were positive for *B. burgdorferi* (59.52%) and 40/79 sera were positive for *R. rickettsii* (50.63%).

Conclusion

For the first time, the presence of antibodies against these three pathogens is reported in the municipality of Jalpan de Serra, in Queretaro, Mexico. These results highlight the importance of preventive medicine and public health in the control of zoonotic diseases transmitted by ticks in the regions of the country where vectors of these diseases are present.
Sensitivity of two-tiered Lyme disease serology in children with an erythema migrans lesion

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**Introduction**

While an erythema migrans (EM) lesion is diagnostic of early Lyme disease, Lyme disease serology may be negative as the patient may not have had time to develop an immune response. The sensitivity of Lyme disease serology for Lyme disease in children with an EM lesion has not been rigorously evaluated.

**Methods**

We performed a prospective eight-center study of children 1 to 21 years of age presenting to a Pedi Lyme Net emergency department for evaluation for Lyme disease between June 2015 and December 2021. For this study, we selected children with a single or multiple EM lesion(s) measuring at least 5 cm in diameter on examination who had Lyme disease serology obtained. Positive two-tier serology was defined with a positive or equivocal first tier enzyme immunoassay (EIA) followed by a positive supplemental immunoblot. We report the sensitivity of two-tier Lyme disease serology overall and for children with single and multiple EM lesion(s).

**Results**

We enrolled 158 children with an EM lesion of which 152 (96.2%) had two-tier Lyme disease serology obtained. Overall, 94 (61.8%) had a single and 58 (38.2%) had multiple EM lesions. The median denominator of the largest EM lesion was 6 cm (interquartile range 5-10 cm). Overall, the sensitivity of two-tier two-tier Lyme disease serology was 50.7% (95% CI 42.4-58.9%), and lower with a single (36.2%, 95% CI 26.5-46.7%) than multiple EM lesions (74.1%; 95% CI 60.9%-84.8%; difference 38.1%, 95% CI 21.9-51.1%).

**Conclusion**

Lyme disease serology is frequently negative in children with early Lyme disease, especially with a single EM lesion. The diagnosis of early Lyme disease should rely on careful physical examination rather than serology.
P111 - Frequency of tick-borne coinfections in children with suspected Lyme disease

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Introduction
In endemic regions, Ixodes species ticks can transmit Borrelia species as well as other tick-borne pathogens. The frequency of tick-borne infections and co-infections in children with suspected Lyme disease is poorly characterized due to shortcomings of current microscopy and serologic diagnostic methods. Importantly, this creates clinical uncertainty about the optimal approach to diagnosis and management of suspected tick-borne infections in children.

Methods
We performed a prospective eight-center study of children 1 to 21 years of age presenting to a Pedi Lyme Net emergency department for evaluation for Lyme disease between June 2015 and December 2021. We defined a case of confirmed Lyme disease based on presence of erythema migrans (EM) lesion or positive two-tier serology in the appropriate clinical context. For this study, we selected Lyme cases with either a single EM lesion or neurologic Lyme disease (facial palsy and/or meningitis) matched by age, gender and clinical center to clinical mimics (i.e. children with facial palsy or meningitis but negative two-tier Lyme disease serology). We also included children with a recent tick bite without symptoms of tick-borne infection. Using biobanked whole blood research samples, we performed a research multiplex high definition polymerase chain reaction (HDPCR) panel (ChromaCode, Carlsbad, CA) to test for 8 bacterial and 1 protozoan tick-borne pathogens. We report the frequency of tick-borne co-infections in children with Lyme disease and matched clinical mimics.

Results
Of the 617 study patients, 306 (49.6%) had a single EM lesion or neurologic Lyme disease, 302 (48.9%) clinical mimics and 9 (1.5%) had a recent tick bite without evidence of infection. The median patient age was 10 years (interquartile range 6-14 years) and 370 (59.9%) were male. To date, we have run 183 multiplex PCR panels of which 4 (2.2%) failed sample quality checks. Of the 179 completed multiplex PCR panels to date, 6 children with early neurologic Lyme disease had a previously unknown tick-borne pathogen identified using the HDPCR panel (2 Anaplasma phagocytophilum and 4 Babesia microti) and 1 had B. burgdorferi/B. mayonii detected. Tick-borne coinfections were identified more frequently in children with confirmed Lyme disease (7.1% Lyme disease vs. 0% clinical mimics; p=0.07). Clinically, all 4 with Babesia spp. and 1 with A. phagocytophilum were treated with antibiotics ineffective for this coinfection.

Conclusion
A significant minority of children with suspected Lyme disease also had other tick-borne infections identified by multiplex HDPCR panel. Reliance on traditional diagnostic methods and clinical presentation my underdiagnose or misdiagnose these infections leading to ineffective
antibiotic therapy. Further study is needed to identify children at highest risk of tick borne co-infections to guide clinical decision-making.
P112 - Lyme disease social representations after a tick bite: how do patients attribute their post-bite health problems?

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Introduction

The scarce psychosocial literature on people who have been bitten by a tick and eventually get Lyme disease highlights the difficult experience these people have, especially given the controversy surrounding Lyme disease.

Aims

The qualitative and exploratory study ‘C18-48 Quali-Explo-PIQTIQ’ (2019), primarily investigated the social representations of tick bites and of Lyme disease in patients bitten by a tick. In particular, we focused on the role of loved ones in orienting patients’ Lyme disease diagnosis and/or auto-diagnosis.

Methods

Semi-structured and exploratory interviews were conducted with 24 patients bitten by ticks in three French medical units. Patients had been clinically categorized as symptomatic, asymptomatic and diagnosed with Lyme disease. Thematic and patient trajectory analyses were performed on the collected data. Trajectory analysis aimed to valorize the temporal perspective of participants’ discourses on their experience, starting from the tick-bite.

Results

We focused on the first stage of the patient trajectory (i.e. the ‘pre-diagnostic work’), beginning after the tick bite and the possible emergence of health problems, and ending before medical consultations. Patients attributed non-specific and non-visible health problems following their tick bite either to the bite itself (sequelae or active Lyme disease) or to other factors, for example another disease, a stressful situation or their age. For a minority of the participants, these attributions analytically corresponded to different self-identified health statuses (i.e., ill, healthy, cured). Other participants were unsure about their health status and lived a condition of uncertainty about it. Furthermore, loved ones played a role in influencing patients’ attributions of health problem, thereby orienting the patient trajectory.

Conclusion

Using different levels of analysis presented by Doise (1986), we were able to define the pre-diagnostic work performed by patients as dynamic, interactional and social. So, auto- and pre-diagnosis is shaped with and by the loved ones, who participated to the health problems identification and attribution. Using both social representation theory and attribution theory, we were able to highlight how patients’ gave sense - albeit lay - to their experience through social interactions. Consequently, new health behavior, especially concerning tick prevention, can be adopted by patients, depending on the trajectory they had lived. Healthcare providers should take into account the existence of pre-diagnostic work performed by patients and their loved ones, as this work could orient the subsequent ‘diagnostic work’ stage of the patient trajectory and the physician-patient relationship.
Introduction
Since Lyme Disease was first mentioned in Dutch medical literature in 1987, its diagnosis and treatment has been surrounded by controversy. Three decades later, 25,000 people contract Lyme Disease each year, 1000 - 2500 of whom suffer persistent symptoms. Although evidence which claims that the Borrelia Burgdorferi spirochete can be transmitted during pregnancy has been available since the mid-1980s, there is no information on the prevalence and incidence of Congenital Lyme Disease in the Netherlands. In a survey conducted by the Dutch Lyme Patients Association (DLPA) in spring 2019, questions about Congenital Lyme Disease were included for the first time.

Aims
The Dutch Lyme Patients Association is keen to gain insight into the number of women and children living with this condition and their quality of life and aims to improve their position. Since two years the DLPA therefore organizes a support group for members of the DLPA and others with concerns around this topic.

Methods
The research methodology is a combination of a population survey among DLPA members, a literature study and a documented case.

Results
Though there are no Dutch publications on Congenital Lyme Disease, cases of Congenital Lyme Disease have been mentioned by 58 people in our survey.

Conclusion
A vicious circle becomes increasingly visible: when Congenital Lyme Disease is considered to be very rare, pregnant women receive no monitoring during pregnancy and their newborns who are potentially at risk will not be tested or monitored during their first year, rarely resulting in a diagnosis of Congenital Lyme Disease. The incidence and prevalence of Congenital Lyme Disease remains unknown, and such children's health and development is not monitored. Six recommendations for improving the position of children with Congenital Lyme Disease and their mothers will be presented.
P114 - 16S Metagenomics for Detection, Surveillance, and Discovery of Bacterial Tickborne Pathogens in the United States

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Introduction

Bacterial tickborne diseases including Lyme disease, relapsing fever, anaplasmosis, ehrlichiosis, spotted fever rickettsioses, and tularemia represent a significant and increasing public health threat in the United States. Shortcomings of existing tickborne disease detection methods include reliance on serologic or targeted molecular methods that test for a single agent or are limited to detection of known agents. This diagnostic challenge is compounded by the etiologic diversity and number of known bacterial infectious agents which cause tickborne diseases. To address these challenges, we developed and validated a broad, sequence-based single assay capable of detecting and providing strain level characterization of all bacterial tickborne pathogens.

Methods

A high-throughput 16S rRNA sequence-based metagenomics approach was developed for direct detection of tickborne bacterial pathogens in blood, cerebrospinal fluid, synovial fluid, and tissue specimens. The V1-V2 region of the 16S rRNA gene is amplified from human specimens, multiplexed, and 384 amplicon pools are subjected to next-generation sequencing using the Illumina MiSeq, followed by assignment of taxonomic predictions. Background taxonomic IDs established by testing healthy human donor blood samples and molecular grade water samples are subtracted from taxonomic predictions for all clinical specimens. To date, >30,000 residual clinical specimens, originally submitted for PCR testing of seven different bacterial tickborne pathogens, have been tested from US patients nationwide.

Results

Overall, 1,965 specimens (6.4% of total) were positive for bacterial tickborne agents, with Rickettsiales (62.25%) and Spirochateales (11.05%) as the most prevalent taxa identified. Eleven known tickborne bacteria were detected in clinical specimens as well as six Spirochateales or Rickettsiales species not known to be associated with human illness, two of which have been previously identified in ticks. No other bacterial taxa were present at similar prevalence; six known non-tickborne bacterial pathogens were detected at low frequency. Borrelia, Anaplasma, Ehrlichia, Rickettsia, and Francisella taxonomic predictions were unique to clinical specimens. Only two B. burgdorferi sensu lato genospecies, Borrelia burgdorferi and Borrelia mayonii, were detected in clinical specimens. B. burgdorferi infections were detected in specimens from the upper Midwest, Northeast, and mid-Atlantic states, whereas B. mayonii infections were localized to the upper Midwest. Seven blood specimens yielded detection of two tickborne pathogens, including five Anaplasma phagocytophilum, B. burgdorferi co-infections.

Conclusion
Our results demonstrate 16S rRNA metagenomics can broadly detect both known and novel bacterial tickborne pathogens in clinical specimens from patients suspected of tickborne illness. The number of bacterial pathogens detected by 16S metagenomics represents a 200% increase compared to initial PCR testing of these clinical specimens. The identification of novel Spirochetales and Rickettsiales bacterial agents demonstrates potential for rapid acceleration of pathogen discovery. In the future, this type of methodology could also simplify test ordering by clinicians to improve early diagnosis of bacterial tickborne diseases.
P116 - Borrelia burgdorferi sensu lato DNA in serum samples of patients with ocular manifestations

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Introduction
Lyme borreliosis (LB) is caused by spirochaetes from the Borrelia burgdorferi sensu lato (s.l.) complex and transmitted to humans by the bites of ticks of the genus Ixodes. The most common clinical manifestation is erythema migrans (EM), an expanding skin redness that usually develops at the site of a tick bite. Pathogenic Borrelia species can spread to other tissues and organs, resulting in manifestations that can involve nervous system, joints, heart and skin. Eye involvement appears to occur very rarely and is associated with other clinical manifestations of LB or it can be the sole manifestation of LB. Primary inflammation of the eye caused by Borrelia includes a wide range of manifestations (e.g. in stage 1: conjunctivitis, episcleritis, scleritis, in stage 3: keratitis, uveitis (anterior, intermediate, posterior, retinal vasculitis, neuroretinitis, panuveitis)), while secondary effects are results of extra-ocular manifestations of infection (e.g. orbital myositis, neuro-ophthalmological manifestations (papillitis, optic neuritis, cranial nerve palsies)).

Aim
The data on the epidemiology of LB in Serbia and the diversity of the Borrelia species causing LB were not available up to date. Therefore, the aim of this pilot study was to reveal the species causing LB in Serbian population with emphasis on the ophthalmological manifestations.

Methodology
The blood samples were taken from 49 patients with suspected LB and collected at the University Clinical Centre of Serbia - Clinic for eye diseases. After isolation of serum, samples were checked for the presence of antibodies against B. burgdorferi s.l. by commercial ELISA assay and all were negative. DNA was extracted from sera, and rrf-rrl rDNA intergenic spacer was amplified by nested PCR. Afterwards, sequencing of obtained amplicons was performed, and sequences were compared with those previously published in the GenBank® database using the BLAST tool.

Results
The presence of DNA of B. burgdorferi s.l. species was detected in 8.2 % (4/49) of patients. Two patients were hospitalized at Clinic for eye diseases due to diagnoses anterior uveitis and in one case retinal detachment and in another case chorioretinitis (symphatetic ophthalmia). The other two patients were not hospitalized, but diagnosed with fibrinous anterior uveitis, syndromata cephalalgica alia and facial nerve disorders at the Neuro-ophthalmology cabinet of Clinic for eye diseases. According to BLAST analysis, three samples aligned with Borrelia afzelii and one sample with Borrelia garinii.

Conclusion: This is the first finding of B. burgdorferi s.l. DNA in serum samples from patients in Serbia with ocular manifestations. Since, onset of eye involvement of LB is
difficult to assess due to often insidious onset and the interval from EM may range from a few days to years, we can assume that *Borrelia* species can be responsible for ocular manifestations of LB in Serbia.
P116 - Oligoclonal bands in cerebrospinal fluid from patients with neuroborreliosis – BorrSci

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**Introduction**

Oligoclonal bands (OCB) in the cerebrospinal fluid (CSF) indicate chronic or acute intrathecal inflammation. We explore the presence and the variance of OCB in patients with neuroborreliosis (NB) by using capillary isoelectric focusing.

**Methods**

We selected 45 out of 121 patients with NB included in the study “Six versus two weeks treatment with doxycycline in Lyme neuroborreliosis - BorrSci”. CSF and serum were collected at the time of diagnosis and at six months follow up. All samples were analysed by capillary isoelectric focusing (CIEF) on the Peggy Sue platform (Proteinsimple). In CIEF, immunoglobulins are separated by charge before immobilization at their isoelectric point. OCBs were counted manually by a trained technician. Samples with more than ten OCBs were designated as >10 OCBs. Based on the OCB patterns, patients were classified in six groups:

Type 1: Normal findings, no bands in CSF.

Type 2: Oligoclonal bands in CSF, not in serum, indicative of intrathecal immunoglobulin synthesis.

Type 3: Oligoclonal bands in CSF and serum. Some OCBs in CSF are not present in serum, indicative of intrathecal immunoglobulin synthesis.

Type 4: Identical oligoclonal bands in CSF and serum, indicative of systemic inflammation.

Type 5: Monoclonal bands in CSF and serum, this may be due to monoclonal gammopathy.

Type 6: A single band in CSF, but not in serum, uncertain clinical significance.

**Results**

At inclusion, none of the patients had normal findings (type 1). Thirty of the patients had type 2 or type 3, indicating intrathecal production of immunoglobulin G (IgG). The number of OCBs among these patients ranged from 2 - >10. One patient had type 4, and one patient was classified as both type 2 and type 5. Four patients had type 5, and nine patients had type 6. All 45 patients had significant pleocytosis (leucocytes ≥ 5/mm³) in CSF at inclusion, ranging from 7 - 678 leukocytes/mm³ (mean 202 cells, median 123 cells). Six months after treatment, 42 of the 45 patients had one specific oligoclonal band at isoelectric point 8.6. Seventeen patients had pattern type 2, and one patient pattern type 3, with a span from 2 - >10 OCBs. At six months, six patients had pleocytosis in CSF, varying from 5-7 leukocytes/mm³.

**Conclusion**

Neuroborreliosis induces intrathecal clonal production of borrelia-specific antibodies. Presence of OCBs in CSF should be followed by an antibody test for Borrelia burgdorferi. None of the patients in this study had pattern type 1, which supports that CIEF is a highly sensitive method.
Introduction
Lyme borreliosis (LB) is a tick-borne disease caused by *Borrelia burgdorferi* sensu lato transmitted by *Ixodes* ticks. Over half a million new cases of LB are diagnosed in the Northern hemisphere each year. Clinical manifestations include early and chronic skin infection as well as disseminated infection such as Lyme arthritis, neuroborreliosis, or carditis. Current serological tests detecting IgM and IgG antibodies against *B. burgdorferi* are unable to differentiate an ongoing or past infection which is important to determine treatment methods. In this study we investigate IgA seroreactivity to *Borrelia*-specific C6-peptide in early LB patients and its role in host-pathogen interactions.

Methods
IgM, IgG and IgA seroreactivity against C6-peptide was determined in early LB patients (*n*=50, LymeProspect NTR4998) presented with erythema migrans (EM). Serum was obtained at presentation, as well as 6 and 12 weeks after presentation. Opsonization of serum IgM, IgG and IgA to (a mixture of) *B. burgdorferi, afzelii* and *garinii* was determined by flow cytometry. IL-6 and IL-1β cytokine release by monocytes was measured after stimulation with serum-opsonized *B. burgdorferi, afzelii* and *garinii* with either pooled human serum of healthy individuals or EM patients. To specify the inflammatory effect of antigen-specific IgA antibodies, similar experiments were performed with blockage of the IgA Fc receptor.

Results
C6 IgM/IgG and IgA antibodies were present in early LB EM serum samples at presentation in approximately 32% and 24% of the patients respectively, with no significant changes in follow-up samples at 6 weeks or 12 weeks. Although the level of binding of IgM, IgG and IgA antibodies to *B. burgdorferi, afzelii* and *garinii* varied between LB patients, opsonization was detected at all three time points in positive patients. Human monocytes stimulated with IgA-opsonized *B. burgdorferi, afzelii* and *garinii* released significantly higher amounts or pro-inflammatory cytokines IL-1β and IL-6 compared to stimulation with non-opsonized or IgG-opsonized spirochetes. Blocking the IgA Fc receptor on monocytes significantly reduced IL-1β release after stimulation with *B. burgdorferi, afzelii* and *garinii* opsonized with serum of LB patients.
Conclusion

IgA seroreactivity against *B. burgdorferi* s.l. antigens in early LB patients induces a potent pro-inflammatory response in human monocytes which can be reversed by blocking the IgA Fc receptor. This indicates that IgA seroreactivity plays an important role in host-pathogen defense and might be an indication of ongoing inflammation in LB patients. Currently we are investigating whether IgA seroreactivity to known and novel identified *B. burgdorferi* s.l. antigens in treated and untreated late LB patients, presenting with the chronic skin manifestation acrodermatitis chronica atrophicans (ACA), can be used to differentiate between an ongoing or past infection.
P118 - Feasibility Assessment of a New Automated VlsE1/pepC10 IgG/IgM Chemiluminescent Immunoassay for Detecting Borrelia-Specific Antibodies from North American and European Serum Donors

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Introduction
Lyme borreliosis is the most common vector-borne illness in North America and Europe, caused by Borrelia burgdorferi sensu lato. So called ‘next-generation’ serology assays theoretically represent an acceptable option for testing North American and European patient populations, due to utilizing recombinant proteins and/or peptides containing amino acid sequences conserved across the different Borrelia species. The aim of this study was to assess the feasibility of detecting Borrelia-specific antibodies from North American and European serum donors, using a newly developed VlsE1/pepC10 IgG/IgM chemiluminescent immunoassay (CLIA) and automated analyzer.

Methods
North American cohort: 92 serum samples were provided by the U.S. Centers for Disease Control and Prevention (32 cases, 60 controls). The CDC provided first-tier screening and Western blot data derived from their own testing. Additional Stage 2 Lyme disease samples, and disease-matched controls, were provided by the Lyme Disease Biobank (13 cases, 14 controls). The LDB provided first-tier screening data derived from their own database. European cohort: 40 serum samples were provided by Amsterdam University Medical Centers (20 cases, 20 controls). AUMC selected seropositive and seronegative cases and controls and provided the interpretation of the first-tier screening and Western blot data derived from their own testing. All samples were assayed by the VlsE1/pepC10 CLIA at ZEUS Scientific. For the LDB samples, Western blots were also freshly performed on first-tier positive samples. Putative sensitivity and specificity values were calculated for the following: [VlsE1/pepC10 CLIA data] versus [Existing screening assay data] or [VlsE1/pepC10 CLIA data + Western blot data] versus [Existing screening assay data + Western blot data] interpreted as standard two-tiered testing (STTT) algorithms.

Results
North American cohort: [VlsE1/pepC10 CLIA, Sensitivity = (38/45) = 84.44%, Specificity = (7/74) = 90.54%; Existing screening assay data, Sensitivity = (37/45) = 82.22%, Specificity = (17/74) = 77.03%], STTT - VlsE1/pepC10 CLIA + Western blots, Sensitivity = (33/45) = 73.33%, Specificity = (0/74) = 100.00%; STTT - Existing screening assay data + Western blots, Sensitivity = (28/45) = 62.22%, Specificity = (2/74) = 97.30%]. European cohort: [VlsE1/pepC10 CLIA, Sensitivity = (20/20) = 100.00%, Specificity = (2/20) = 90.00%; Existing screening assay data, Sensitivity = (19/20) = 95.00%, Specificity = (5/20) = 75.00%], STTT - VlsE1/pepC10 CLIA + Western blots, Sensitivity = (15/20) = 75.00%, Specificity = (2/20) = 90.00%; STTT - Existing screening assay data + Western blots, Sensitivity = (15/20) = 75.00%, Specificity = (2/20) = 90.00%

Conclusion
These preliminary studies demonstrate that the newly developed VlsE1/pepC10 IgG/IgM CLIA, in conjunction with a new automated analyzer, yields sensitive and specific results from North
American and European serum donors. Additional studies using larger sample cohorts from each geographic region are warranted towards further validating the performance of this new assay alone, as well as part of various two-tiered testing approaches.
P119 - Lyme arthritis. A rare but important cause of mono- or oligo arthritis in Denmark: A case series of Lyme arthritis in three Danish male patients

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Introduction
Lyme arthritis (LA) is a rare late manifestation of Lyme disease (LD) in Denmark with an unknown incidence. It is caused by spirochetes within the Borrelia burgdorferi sensu lato complex (B.b.s.l.). The species Borrelia burgdorferi sensu stricto (B.b.s.s.) is known for its tendency to cause LA, especially in North America. In Europe, including Denmark, the number of co-existing pathogenic B.b.s.l. species are more diverse; the main cause of LA are still thought to be B.b.s.s, but other species could be responsible. Because of the low incidence of LA in Denmark, no routine diagnostic procedure is in place.

Method and Results
We describe a case series consisting of three Danish male patients diagnosed with LA within a half year between 2021-2022 in Esbjerg, Denmark. All patients debuted with recurrent knee joint swelling, yet one patient also had arthritis in the upper extremities. Two patients had a history of tick bite, none had erythema migrans. The diagnosis was delayed because of lack of attention of LA as a potential differential diagnosis. The diagnosis was established based on B.b.s.l. anti-IgG antibodies in patient serum and detection of B.b.s.l. DNA by real-time PCR in synovial fluid. The patients received intravenous cefuroxime 2 grams for 28 days. One patient was re-treated because of recurrence of joint swelling and positive PCR result in a different joint after completing the first antibiotic regimen. One patient has fully recovered and have shown no sign of arthritis for more than 8 months. The remaining two patients are in remission on methotrexate.

Conclusion
This case series demonstrates that LA is an important differential diagnosis in persistent or intermittent mono- or oligo arthritis despite a presumed low incidence in Denmark. It could be speculated that the low incidence of LA is caused by a lack of attention to B.b.s.l. as a possible explanation for mono- or oligo arthritis. Assessment of the patients tick risk behavior, B.b.s.l. antibody status and/or assessment of the presence of B.b.s.l. DNA in synovial fluid by PCR methods is not commonplace. Obviously, insufficient examinations can lead to a falsely assumed low incidence. Our case series suggest that attention to LA as a possible differential diagnosis is justified and an effort for improving the diagnostic facility should be made. It highlights the need for clinical centres invested in late manifestations in LB patients, to ensure a correct diagnosis and sufficient treatment.
P120 - Detection of Borrelia burgdorferi sensu stricto cell-free DNA in human plasma samples for improved diagnosis of early Lyme borreliosis

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Background
Laboratory confirmation of early Lyme borreliosis (LB) is challenging. Serology is insensitive during the first days to weeks of infection, and blood PCR offers similarly poor performance. Here, we demonstrate that detection of Borrelia burgdorferi (B.b.) cell-free DNA (cfDNA) in plasma can improve diagnosis of early LB.

Methods
B.b. detection in plasma samples using unbiased metagenomic cfDNA sequencing performed by a commercial laboratory (Karius Inc.) was compared with serology and blood PCR in 40 patients with physician-diagnosed EM, 28 of whom were confirmed to have LB by skin biopsy culture (N=18), seroconversion (N=2) or both (N=8). B.b. sequence analysis was performed using investigational detection thresholds, different from Karius’ clinical test.

Results
B.b.cfDNA was detected in 18/28 patients (64%) with laboratory-confirmed EM. In comparison, sensitivity of acute-phase serology using modified two-tiered testing (MTTT) was 50% (P=0.45) and sensitivity of blood PCR was 7% (P=0.0002). Combining B.b.cfDNA detection and MTTT increased diagnostic sensitivity to 86% in this cohort, significantly higher than either approach alone (P<0.04). B.b.cfDNA sequences matched precisely with strain-specific sequence generated from the same individual’s cultured B.b. isolate. B.b.cfDNA was not observed at any level in plasma from 684 asymptomatic ambulatory individuals. Among 3000 hospitalized patients tested as part of clinical care, B.b.cfDNA was detected in only two individuals, both of whom had clinical presentations consistent with early LB.

Conclusions
This is the first report of B.b.cfDNA detection in early LB and a demonstration of potential diagnostic utility. The combination of B.b.cfDNA detection and acute-phase MTTT improves clinical sensitivity for diagnosis of early LB.
P121 - Knowing the entire story – a focus group study on patient experiences with chronic Lyme-associated symptoms (chronic Lyme disease)

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Introduction
Healthcare providers frequently struggle to provide effective care to patients with chronic Lyme-associated symptoms (chronic Lyme disease, CLD), potentially causing these patients to feel misunderstood or neglected by the healthcare system. This study is the first to use a combined medical and communication science approach to assess patients’ experiences with CLD-related care, and provides potential ways to improve communication with these patients, irrespective of the aetiology of their symptoms.

Methods
Informed by the principles of ‘clean language’, we conducted focus groups with self-identified CLD patients (N=15). We asked participants about their experiences with CLD and CLD-related healthcare. We performed thematic analyses using a bottom-up approach based in discourse analysis. We also sought to identify specific types of verbalizations (repertoires) across themes.

Results: Participants reported a heterogeneous set of CLD-associated symptoms, which they frequently labelled as ‘invisible’ to others. Their illness significantly affected their daily lives, impacting their work, social activities, relationships with loved ones, hobbies and other means of participating in society. Negative experiences with healthcare providers were near-universal, also in patients who had had CLD-associated symptoms for relatively short periods of time. Participants wished their healthcare providers were more specialized in LD/CLD, but at the same time wanted them to have a more holistic view. Verbalizations were notable for frequent use of communicative modes that implicitly create common ground between participants and that give a certain validity to personal experiences. These include use of impersonal ‘you’ (‘You try to keep a job, but there is little you can do’), rather than ‘I tried to keep a job, but there was little I could do’) and other forms of presupposition.
Conclusion: We find that CLD patients experience significant symptoms, for which they only rarely find adequate relief from conventional medical practitioners. This study identifies various repertoires in these patients’ shared experiences, such as a feeling of abandonment and lack of understanding by the medical system, feelings of loss with respect to their previous health, and the idea that they might have been better off had they been diagnosed sooner. Working with these repertoires will enable healthcare providers to establish a shared perspective with their CLD patients, thereby engaging in more fruitful doctor-patient communication. We hypothesize that these findings are not unique to CLD, but may also be applicable to other contested illnesses, such as Long COVID.
Introduction
Tick-Borne Encephalitis Virus infection and Borrelia burgdorferi infection are emerging diseases, but only limited information on Tick-Borne Encephalitis Virus (TBEV) and Borrelia burgdorferi (Bb) co-infection is available. Reports about dual infection are rare, maybe because after the diagnosis of one disease, clinicians do not consider the other. However, TBEV-Bb co-infection has to be more frequently considered in endemic areas for tick-borne-diseases, since there are two different ways of treatment.

Methods
We describe a series of four patients with TBEV-Bb co-infection, admitted to the Infectious Disease Unit of Belluno, a city of North Eastern Italy, characterized by a very dense tick population and a high prevalence of TBEV and Bb.

Results
Of the four patients with TBEV and Bb co-infection, two reported a clinical biphasic course, characterized by a first phase with aspecific symptoms and a second phase with neurological symptoms with an asymptomatic interval of five days. The other two patients had a monophasic course, with neurological symptoms. Each one had symptoms correlated to both TBEV and Bb, such as fever, headache, arthromyalgia, asthenia and neurological features (i.e. meningism, disorientation, ataxia, tremors and limb palsy). Only two persons presented migrans erythema, which is typical of Bb infection. Cerebrospinal fluid (CSF) was always characterized by pleocytosis (prevalence of lymphocytes). In relation to full blood count examination, patients presented thrombocytopenia, lymphocytopenia or neutrophilia. In one case high levels of transaminases and bilirubin were detected. Normal level of CRP was always observed. In three cases, IgG and IgM for Bb were firstly negative, then positive: this is the reason why diagnosis of borreliosis was based primarily on migrans erythema in two cases, while on Bb DNA identification in CSF in one case. IgG and IgM for TBEV were negative only during the first phase of the biphasic course, afterwards positivity was detected. Electroencephalogram (EEG) always showed generalized abnormalities; after one month follow up, the EEG was normal in each one. Bb infection was treated with antibiotic therapy, while TBEV infection with symptomatic treatment. No one died from co-infection, but all patients had sequelae at 1-month follow-up, such as asthenia, limb palsy and headache.

Conclusion
TBEV and Bb co-infection should be considered in regions were both pathogens are endemic, even if the initial clinical symptoms suggest an infection with only one of the pathogens. There has been no evidence of unusual manifestations, but the limited number of the reported cases makes it impossible to decide if double infection leads to a more severe manifestation than a single infection with TBEV or Bb. Further research is needed to determine how these organisms interact during transmission to the host and the impact on the clinical course of the affected people.
P123 - Lyme neuroborreliosis with Neoehrlichia mikurensis co-infection – a case report

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Introduction

While Lyme neuroborreliosis (LNB) is a well-established infection in northern Europe, infection with the tick-borne bacterium, Neoehrlichia mikurensis is relatively new and scarcely described in the scientific literature. Little is known about co-infections with Borrelia burgdorferi s.l. and N. mikurensis.

Case report

A 70- years old man without significant co-morbidities presented with progressing paresis of his left leg. He had been complaining of radicular pain for 3 months leading up to admission and had undergone extensive investigations without results. His symptoms also included myalgia, night sweats, and an unintended weight loss of 6 kg. He had no history of tick-bite or a rash compatible with erythema migrans, but was active in his garden and spent time in the local forest.

At hospital admission, he was found to have neurogenic pain primarily in the left lower extremity accompanied by reduced strength and a foot drop. His blood tests revealed an elevated white blood count of 14 x 10^9 (normal range (NR) 3.5-8.8), slight anemia with hemoglobin 7.6 mmol/L (NR 8.3-10.5 mmol/L), thrombocytosis of 525 x10^9 (NR 150-350 x10^9), elevated creatine kinase 466 U/L (NR 40-280 U/L) and a C-reactive protein of 16 mg/L (< 6 mg/L). Due to hoarseness, an ear-nose-throat examination was performed, and revealed no abnormalities. He was monitored due to continuous sinus tachycardia, no other cardiac arrhythmias were found. A lumbar puncture revealed an elevated white blood cell count of 48 x10^6 (98% mononuclear), elevated protein 1.92 g/L. Ceftriaxone was initiated on suspicion of LNB. Intrathecal production of B. burgdorferi IgG antibodies confirmed the diagnosis and the antibiotic regimen changed to doxycycline. The total antibiotic treatment duration was 21 days. A re-lumbar puncture 3 weeks after treatment showed a white blood cell count of 7x10^9 and normalized protein. MRI of cerebrum and PET/CT were both normal, ENG/EMG showed subacute asymmetric sensomotoric changes in both lower extremities.

A blood sample from the time of lumbar puncture was later tested PCR positive for N. mikurensis with a cycle threshold value of 37.

At follow-up 12 months after treatment, the paresis had remitted. His primary complaint was continuous neuropathic pain, but he had regained his habitual weight and all blood abnormalities were normalized.

Discussion

While his symptoms were typical of LNB, our patient displayed more pronounced blood discrepancies compared with what is normally found in LNB patients. Myalgia, night sweats, anemia, and the elevated inflammatory markers might be representative of the N. mikurensis co-infection. Fortunately, N. mikurensis is like B. burgdorferi sufficiently treated with doxycycline. While asymptomatic N. mikurensis infection is previously described in both immunocompromised
and immunocompetent patients, more data is warranted on the clinical implications of co-infections of *B. burgdorferi* and *N. mikurensis*. 
P124 - Can an educational intervention in general practice improve the management of Lyme borreliosis?

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Introduction
In Denmark, the general practitioners (GPs) have a gate-keeping role, and are the first medical professionals to see the majority of Lyme borreliosis (LB) patients. Prior studies have shown lack of knowledge about Lyme neuroborreliosis (LNB) symptoms and incorrect use and interpretation of Serum-Borrelia burgdorferi antibodies among Danish GPs, as well as a delay in referral of LNB patients to hospital.

Aims
Our aims with this study were to improve the following five outcomes in LB patients through an educational intervention in general practice;

- increase the number of hospital referrals on suspicion of LB
- increase the number of cerebrospinal fluid (CSF) tests examined for B. burgdorferi antibody index
- decrease the number of Serum-B. burgdorferi antibody tests ordered
- shorten the delay from symptom onset to hospital admission among LNB patients
- increase knowledge about LB among general practitioners

Methods
We performed a prospective non-blinded non-randomized intervention trial on the island of Funen, Denmark. The intervention included oral and written education about LB, and was carried out in areas with an LNB incidence ≥ 4.7/100.000 between 22.1.2019 and 7.5.2019. Results were compared between the intervention-group (49 general practices) and the remaining general practices in Funen (71 practices) in the two years before and the two years after the intervention.

Results
In the study period, 196 patients from Funen were referred to hospital on suspicion of LB; a 28.9 % increase in the intervention-group post intervention compared with a 59.5 % increase in the control-group (p=0.47). The number of CSF-Borrelia-antibody index tests increased 20.8 % in the intervention-group post intervention, compared with 18.0 % in the control-group (p=0.68). The number of ordered Serum-B. burgdorferi antibody tests declined 43.1 % in the intervention-group post intervention, while it declined 34.5 % in the control-group (p=0.30). In all, 25.1 % had presence of Serum-B. burgdorferi antibodies. We found no difference in LNB pre-hospital delay before and after the intervention or between the two groups (p=0.21). The GPs from the
intervention-group performed significantly better on a follow-up questionnaire compared with GPs from the control-group (p=0.02).

**Conclusion**
We found an overall improvement in LB awareness and referrals among general practitioners over time, but we could not show any effect of the intervention on clinical outcomes of LNB. More efficient approaches are needed to influence clinical behavior and thereby reduce the pre-hospital delay of LNB patients.
P125 - High-throughput drug screen to identify enhanced therapeutics that target the zonal peptidoglycan synthesis of Borrelia burgdorferi to treat Lyme disease

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Introduction
The current therapeutic regimen for Lyme disease, the most prevalent vector-borne illness in the United States, constitutes an aggressive treatment of nonspecific antibiotics, such as doxycycline. These drugs are associated with harmful patient side effects such as severe gastrointestinal implications and are known to severely disrupt the human gut microbiome. Additionally, upwards of 20% of individuals diagnosed with Lyme disease are still symptomatic six months after receiving treatment, further illuminating the need for enhanced therapeutics. Borrelia burgdorferi has a highly unique growth pattern in that the cell elongates through the synthesis of peptidoglycan at distinct zones that are spatially and temporarily regulated.

Objections/Aims
We hypothesize that more effective therapeutics exist but have yet to be discovered due to a lack of high-throughput screening techniques.

Methods
Here, we developed a high-throughput screening methodology to quantify and identify changes in Borrelia burgdorferi mode of growth upon drug treatment. We investigated 467 unique FDA-approved drugs to analyze their potential efficacy against eradicating Borrelia burgdorferi in vitro.

Results
Microtiter growth assays, coupled with quantitative microscopy using HADA, a fluorescent tracer that monitors peptidoglycan biogenesis, identified top performing compounds. By taking advantage of chemical probes, we were able to define the mechanism that underlies the specificity of the efficacious candidates. At a range of up to 45 times less than the effective concentration of doxycycline used to treat Lyme disease, we identified several antibiotic drugs from the beta-lactam, cephalosporin, macrolide, and tetracycline classes that demonstrated deleterious effects on Borrelia burgdorferi cell growth, survival, and morphology. Importantly, the same concentration of compounds had little to no effect on the growth of numerous other bacteria tested. Minimum inhibitory concentration calculations, in conjunction with colony forming, and live infection studies also identify and validate top performing drugs.

Conclusions
These studies identify new candidate compounds to specifically and effectively treat Lyme disease.
P126 - Novel Flow-based Immunosorbent Profiling (FLIP) of Borrelia burgdorferi Reveals IgE as a potential mediator of pathological allergy in Lyme disease

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Introduction
Lyme disease, caused by a bacterial infection with Borrelia spp., is the most common and rapidly growing vector-borne infectious disease in the United States and Europe. High variability in disease burden among Lyme patients suggests that individual immune responses may be key drivers of clinical presentation and patient outcomes.

Methods
We developed a high-throughput method called FLow-based Immunosorbent Profiling (FLIP) for high resolution analysis of the antibody isotypes and subtypes specifically bound to B burgdorferi. This method uses live bacteria as bait for an immunosorbent assay in a flow cytometry based profiling platform to precipitate pathogen-specific antibodies out of solution and then conjugate them with secondary fluorescently labeled antibodies to enable full antibody profiling.

Results
This FLIP assay revealed that a subset of Lyme patients, with either acute or persistent symptoms, were producing high concentrations of IgE specific to B. burgdorferi. This IgE response was seen in C3H/HeJ mice, which are susceptible to Lyme disease, but not observed in C57BL/6 mice, which are tolerant to B. burgdorferi. Further, IgE was found to target B. burgdorferi peptidoglycan in both acute and long-term infection models. We then investigated if this IgE response may lead to mast cell activation and release of highly immunogenic effectors into the surrounding tissues. Histologic analysis of mouse Lyme arthritic ankle tissue showed mast cells degranulating at significantly higher rates compared to uninfected controls. Additionally, induced mast cell degranulation by a CD117 antibody exacerbated Lyme arthritis in infected mice.

Conclusion
This data suggests that a subset of Lyme patients may have developed an allergic response to conserved bacterial antigens from a B. burgdorferi infection. Inclusion of IgE reactivity in
diagnostic testing and examination of pathological immune responses to bacterial antigens could assist clinicians in patient care and effective treatments.
**P127 - Tick-borne Disease Patient Experiences with Providers: Recommendations for Improving Quality of Care & Health Outcomes**

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**Introduction**

There is a growing awareness of the need to increase and improve healthcare provider education to more effectively work with patients with a tick-borne disease. It is increasingly documented that tick-borne disease patients often struggle to find a healthcare provider that is knowledgeable about tick-borne diseases and that can effectively recognize, diagnose, manage, and treat their associated symptoms. Prior research shows misdiagnosis, undertreatment or overtreatment, increased healthcare costs and decreased health related quality of life can all result from provider lack of knowledge and understanding of tick-borne diseases.

**Aim:** The current study was designed to explore tick-borne disease patient experiences with providers to develop recommendations for healthcare provider education that aims to improve quality of care and health outcomes for patients with a tick-borne disease.

**Methods**

In 2021, a mixed method survey and 30-minute follow-up interviews were conducted with adults with a diagnosed tick-borne disease to explore their experiences with their providers and their recommendations for improving care. Snowball sampling was used to recruit participants through email and social media sites. Qualitative data was hand-coded and qualitative analysis software (NVivo) was used to explore patterns, frequency, and themes. Codes and themes were discussed among three researchers for intercoder reliability.

**Results**

A total of 434 adults with a diagnosed tick-borne disease participated in the survey and 43 adults participated in an interview. Participants reported contracting at least 1 of 18 tick-borne diseases in 3 continents, 5 countries, 34 American states or Washington DC. Nearly three in five survey participants (59.7%) were misdiagnosed at least once and one in three were diagnosed (33.6%) with at least one other tick-borne co-infection. The most common misdiagnosis for all types of tick-borne diseases combined was Fibromyalgia (N=76), chronic fatigue syndrome (N=59), mental health condition (N=51), and Rheumatoid Arthritis (N=38). Among the qualitative responses, participants often reported having to educate their provider about their tick-borne disease and having to use the internet to learn how to manage their symptoms. Through thematic analysis, 15 recommendations emerged for providers in one of five major theme areas: Patient-provider interactions, diagnosis and testing, management and treatment, individualized care, and education.

**Conclusion**

For many patients with a tick-borne disease; an earlier diagnosis, an individualized care plan and improved patient-provider interactions could have significantly improved their health outcomes and current health status. The tick-borne disease patient recommendations from this study should be useful for developing healthcare provider education and to all providers looking to
improve tick-borne disease patient-provider relationships and health outcomes. Healthcare providers who apply tick-borne disease patient recommendations in their clinical practice may be more likely to be able to prevent misdiagnosis, mismanagement, and mistreatment of a tick-borne disease.
P128 - Lyme Disease Treatment in the United States: Outpatient Prescribing Patterns from a Nationwide Commercial Insurance Database, 2016-2019

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Introduction
Lyme disease (LD) is transmitted by infected Ixodes spp. ticks and is the most common vector-borne disease in the United States and a growing public health problem. The use of non-recommended antibiotic treatments for LD has been associated with adverse effects, including death; however, prescribing patterns have not been examined on a national scale.

Methods
We performed a retrospective analysis of the MarketScan commercial claims database of outpatient encounters from 2016-2019 in the United States. We identified all individuals with a visit that included an LD diagnosis code and prescription within 30 days of the visit for an antibiotic likely to be prescribed for LD. We then categorized individuals as having received recommended or non-recommended treatments. Recommended treatment was defined as treatment with a first- or second-line antibiotic for Lyme disease for no longer than 30 days, no more than 2 episodes within one year, and no more than 4 episodes over the 4-year study period. Descriptive and multivariable analyses were performed to compare characteristics of individuals who received recommended or non-recommended LD treatment.

Results
A total of 107,472 prescriptions for 58,056 unique patients were identified and included in the analysis. Doxycycline was the most prescribed antibiotic, representing 54% of all prescriptions, followed by amoxicillin (14%) and azithromycin (10%). Most individuals met the definition for recommended treatment during the study period (n = 51,297, 88%). Of all individuals, 8% were classified as receiving non-recommended treatment due to the frequency of antibiotic prescriptions during the study period; 7% had prescriptions for non-recommended drugs, such as tetracycline, minocycline, or erythromycin; and 2% of individuals had a prescription written for greater than 30 days. Female gender (aOR 1.83, 95% CI 1.74-1.93) and age 19-45 years (aOR 1.34, 95% CI 1.28-1.41) were significantly associated with being prescribed non-recommended LD treatment. Prescriptions in low-incidence states (aOR 1.98, 95% CI 1.93-2.05) and between September and April (aOR 2.43, 95% CI 2.37-2.50) were also more likely to be non-recommended.

Conclusions
In this population of insured individuals, young and middle-aged women were at the highest risk of receiving non-recommended antibiotic treatment for LD. Non-recommended LD treatment was more likely to be prescribed for patients in areas and during seasons when questing Ixodes ticks are less common, likely representing misdiagnosis or overtreatment of LD. Commercial claims data are useful to examine Lyme disease prescribing patterns in the United States and can identify patient groups at highest risk for non-recommended treatment. These results can inform targeted healthcare provider and patient education efforts.
P129 - Ticking on Pandora's box: a prospective case-control study into ‘other’ tick-borne diseases

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Introduction
Tick-borne pathogens other than Borrelia burgdorferi sensu lato - the causative agent of Lyme borreliosis - are common in Ixodes ricinus ticks. How often these pathogens cause human disease is unknown. In addition, diagnostic tools to identify such diseases are lacking or reserved to research laboratories. To elucidate their prevalence and disease burden, the study ‘Ticking on Pandora’s Box’ was initiated, a collaborative effort between Amsterdam University Medical Center and the National Institute for Public Health and the Environment.

Materials and methods
The study investigated how often the tick-borne pathogens Anaplasma phagocytophilum, Babesia species, Borrelia burgdorferi s.l., Borrelia miyamotoi, Neoehrlichia mikurensis, spotted fever group Rickettsia species and/or tick-borne encephalitis virus cause an acute febrile illness after tick-bite. We aimed to determine the impact and severity of these tick-borne diseases in the Netherlands by measuring their prevalence and describing their clinical picture and course of disease. The study was designed as a prospective case-control study. We included 137 cases - individuals clinically suspected of a tick-borne disease - and 3 matched healthy control groups of approximately 200-300 persons each. The controls consist respectively of a group of individuals with either a tick-bite without complaints, the general population and of healthy blood donors. During a one-year follow-up we acquired blood, urine and skin biopsy samples and ticks at baseline, and additional blood samples at 4 and 12 weeks. At the time of writing of this abstract all participants completed their follow-up. Additionally, participants answered online questionnaires through the national platform Tekenradar.nl, to assess self-reported symptoms, among which the physical and social functioning subscale SF-36, on a 3 monthly basis.

Discussion
We will report preliminary molecular, serological and clinical data at the conference. With this study we hope to provide insight into the prevalence, clinical presentation and disease burden of the tick-borne diseases anaplasmosis, babesiosis, B. miyamotoi disease, neoehrlichiosis, rickettsiosis and tick-borne encephalitis and to assist in test development as well as provide recommendations for national guidelines.
Introduction
Lyme disease is caused by *Borrelia burgdorferi* sensu lato, which is transmitted through tick-bite. Canine Lyme Disease (CLD) is an established clinical entity in the United States. Although it has been shown that dogs can be naturally infected in Europe and develop antibodies against *B. burgdorferi* s.l., the existence of CLD in Europe has been debated. We here present a prospective cohort study in a tick endemic area in the Netherlands with 84 dogs. In addition 31 Bernese Mountain dogs (BMD), known to have robust anti-*Borrelia* antibody responses, were longitudinally monitored and serologically examined.

Methods
A prospective cohort study included a population of several dog breeds which were observed during 7 consecutive half years. At the same time and extending for 20 more years 31 BMD’s were also monitored. Generalized estimating equations (GEE) analysis on the sequential half-year repeated measurements were employed to detect associations, between clinical, immunological and seasonal variables.

Aim
To study the dynamics and associations of whole cell *Borrelia burgdorferi* sensu stricto total IgG, IgG2 and IgG1 antibodies during several tick-seasons.
And to gain insight in the pathogenesis and incidence of CLD in the Netherlands.

Results
We showed a strong association between the clinical signs fever and lameness in time, which in turn was associated with high total IgG and IgG1 titers against *B. burgdorferi* sensu stricto. In line with these findings, the variable IgG1/IgG2 ≥ 0.4, in combination with the intensity of tick-infestation predicted the combination of fever and lameness. Interestingly, the 31 BMD showed seroconversions and persistence of total IgG and IgG1 titers. Although these dogs reacted strongly against the C6 peptide, their tissues tested negative for *B. burgdorferi* s.l. DNA.

Conclusions
These studies show that dogs in the Netherlands, mostly Labrador retrievers and BMD, over the course of multiple tick-infestation seasons, can develop symptoms compatible with CLD. The occurrence of these transient symptoms was strongly associated with high total IgG as well as IgG1 antibody responses against *B. burgdorferi* s.s.. These findings provide important insights into the pathogenesis of CLD and calls for awareness in daily veterinary practice in Europe.
P131 - Burden of Lyme Disease (BOLD) study: manifestations of clinically-diagnosed Lyme borreliosis from active surveillance at 14 general practices in endemic areas in 6 European countries

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Introduction
Lyme borreliosis (LB) is the most frequently reported tick-borne disease in Europe. LB can involve several organ systems and result in a variety of clinical manifestations, however information on the frequency of manifestations of LB in Europe is limited. The BOLD study was launched in endemic areas in six European countries to determine LB incidence and clinical manifestations at potential clinical trial sites for efficacy evaluation of a candidate LB vaccine.

Aims
Assess the frequency of LB manifestations in selected European countries.

Methods
Fourteen primary care practices in LB endemic regions in Czechia, Germany, Poland, Slovakia, Slovenia and Sweden were recruited as study sites. Active surveillance for newly identified suspected LD cases was conducted beginning April to July, and for the remainder of the year 2021. After informed consent, cases were enrolled and interviewed, and their medical records were reviewed for more detailed information on symptoms and medical history. A final clinical diagnosis was assigned by the site investigator (based on suggested predefined BOLD study case definitions, including specific clinical signs, symptoms and laboratory findings if available) after evaluation of each suspect case.

Results
Between Apr 8, 2021 and Dec 31, 2021, there were 435 suspected LB cases of which 352 (81%) were clinically-diagnosed LB cases. Of the clinically-diagnosed LB cases, 269 (76%) were enrolled in the study, and 7 (3%) of these had more than one manifestation. Of the 269 enrolled cases, 183 (68%) presented with erythema migrans (EM), 77 (29%) had Lyme arthritis, 3 (1%) had Lyme neuroborreliosis, 1 (0.4%) had Lyme carditis, and 12 (4%) had other manifestations (e.g. systemic symptoms only).

The proportion of clinically diagnosed LB cases that presented with erythema migrans in each country ranged from 28% in Slovakia to 96% in Sweden. Lyme arthritis was only reported in 4 of 6 countries; 93% of Lyme arthritis cases were reported by Slovakia. Lyme arthritis comprised 71% of clinically-diagnosed LD cases in Slovakia but only 2-5% in the other three countries.
Conclusions
Primary care practices in endemic areas in 6 European countries have launched the BOLD study which will continue until the end of 2022. In 2021, erythema migrans was the most common manifestation of LB followed by Lyme arthritis, for which the frequency appears to vary markedly by country. Additional studies are needed to further characterize LB clinical manifestations in Europe and understand both drivers and potential biases that may account for variations across countries.
Comparison of erythema migrans in patients with or without neurologic involvement

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Introduction
Lyme neuroborreliosis (LNB) is often associated with erythema migrans (EM). Previous reports demonstrated that patients with borrelial meningoradiculoneuritis (Bannwarth syndrome) have longer duration of EM at diagnosis compared to those without neurologic symptoms. The reasons for this difference are not known.

The aim of the present study was to characterize EM in patients with Bannwarth syndrome, and those without. In addition, we wanted to evaluate if the delay in seeking medical attention was unique to patients with Bannwarth syndrome or was it present with other forms of LNB.

Methods
To answer these questions we compared the characteristics of EM in adult patients with or without nervous system involvement. All the patients were assessed at our Lyme borreliosis Outpatient Clinic. Four groups of patients were evaluated: 114 patients with EM and associated meningoradiculoneuritis (Group 1, Bannwarth syndrome); 394 patients with typical EM associated with symptoms/signs suggesting nervous system involvement but no radicular pain, of which 69 had CSF pleocytosis (Group 2, proven LNB, lymphocytic meningitis) while 325 had normal CSF leukocyte counts (Group 3, suspected LNB). These patients were compared with 12384 patients with EM without obvious neurological impairment (Group 4). All patients met the European diagnostic criteria for EM (1). In addition, patients with Bannwarth syndrome (Group 1) and patients with lymphocytic meningitis without radicular pain (Group 2) fulfilled criteria for definite LNB (2).

Results
Compared to EM patients without neurological symptoms, patients with Bannwarth syndrome were older, had a significantly higher frequency of EM on head/neck and trunk, similar frequency on arms, but lower frequency on legs, longer duration of EM prior to diagnosis, and larger diameter of EM. Moreover, patients with Bannwarth syndrome had almost exclusively *Borrelia garinii* isolated from the skin while EM in patients without neurologic symptoms was caused predominantly by *Borrelia afzelii* (~90%); 8% had *B. garinii* infection. When compared to EM patients with *B. garinii* infection but without neurological involvement, those with Bannwarth syndrome and EM reported lower frequency of local as well as constitutional symptoms (other than radicular pain), possibly because the intense radicular pain “masked” other minor local or constitutional symptoms. Similar findings were observed when comparing EM in patients with Bannwarth syndrome to those with suspected or confirmed LNB without radicular pain, suggesting that these distinct EM characteristics are unique to patients with Bannwarth syndrome.

Conclusions
Patients with EM who develop Bannwarth syndrome have several distinct clinical characteristics.
associated with EM. These results point to multifactorial cause for the delay in recognition of EM in patients with Bannwarth syndrome.
**P133 - Relative frequency, the course and the outcome of the major clinical manifestations of early European Lyme neuroborreliosis**

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**Introduction**

European Lyme neuroborreliosis (LNB) encompasses a broad clinical spectrum with Bannwarth syndrome (BS), a painful meningo-radiculoneuritis, being the most typical clinical manifestation. Several other clinical presentations without radiculitis, such as cranial neuritis (most often represented by peripheral facial palsy, PFP), and lymphocytic meningitis, have been recognized, but the information of their relative frequency is limited.

**Aims**

The goal of the study was to ascertain the relative frequency of 3 major clinical manifestations of early LNB in adult European patients and to compare their presentation, course and outcome.

**Methods**

This prospective study comprised patients aged ≥15 years, diagnosed with early LNB between October 2005 and December 2020 at a single center. Borrelial infection was searched for in: 1) patients with radicular pain of recent onset (suspected BS), 2) those with PFP or other cranial nerve involvement, and 3) patients with erythema migrans (EM) and associated neurological symptoms. Confirmed (definite) LNB was attested with: i) cerebrospinal fluid (CSF) pleocytosis and ii) demonstration of borrelial infection by intrathecal synthesis of borrelial antibodies and/or positive CSF borrelia culture and/or the presence or reliable history of recent EM. After 14-day treatment with ceftriaxone or doxycycline patients were followed at regular visits for one year.

**Results**

Of 1405 patients with clinically suspected LNB who presented with one of the three clinical entities and had CSF examination performed, 303 (21.6%) patients fulfilled criteria for confirmed LNB (174 males, 129 females, median age 56 years). Confirmed LNB was demonstrated in the highest proportion in patients with clinically suspected BS (185/286, 64.7%), followed by patients with EM associated with constitutional symptoms suggesting central nervous system involvement (41/213, 19.2%), and patients who presented with cranial neuropathy (77/906, 8.5%). Most frequent clinical presentation in 303 patients with confirmed LNB was meningoradiculoneuritis (BS) (61.1%), followed by PFP or other cranial nerve involvement (25.4%), and EM-associated lymphocytic meningitis (13.5%). Median duration of neurological symptoms before diagnosis was 20 days. Most often symptoms were radicular pain (61.1%), sleep disturbances (56.8%), headache (48.2%), fatigue (37.0%) and malaise (28.4%). Objective signs found at presentation were PFP (45.5%), EM (35.3%), meningeal signs (16.2%), pareses (7.6%), other cranial nerve involvement (2.6%), tremor (2.0%), and borrelial lymphocytoma (1.0%). Intrathecal synthesis of borrelial IgM and IgG antibodies was present in 48.2% and 72.3% of patients, respectively, while borreliae (predominantly *B. garinii*) were isolated from CSF, skin and blood in 9.2%, 30.1% and 1.9% of patients, respectively. The outcome assessed 12 months after antibiotic treatment was favourable in 94.7% of patients and was comparable in the three groups.
Conclusion
BS is the most frequent manifestation of early European LNB, followed by cranial neuropathy and EM-associated lymphocytic meningitis. BS distinguishes from other early LNB manifestations in symptomology, laboratory findings, and etiology.
Factors associated with the diagnostic acceptance and the management satisfaction of the patients experiencing a multidisciplinary management for suspected Lyme borreliosis at 12 months

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Introduction
Because many patients with a suspicion of Lyme borreliosis (LB) experience difficult care paths, the Tick-Borne Diseases Reference Center (TBD-RC) was started in 2017. To our knowledge, there are not any study about the satisfaction of the patients of these new proposed multidisciplinary care paths. The aim of our study was to describe the satisfaction of the patients experiencing a multidisciplinary management for suspected LB, and to identify the determinant factors of their diagnosis acceptance.

Methods
We included all adult patients who were seen at the TBD-RC (2017-2020). Four groups were defined according to the European Study Group for LB and French guidelines: i) confirmed LB, ii) possible LB, iii) Post-Treatment Lyme Disease Syndrome (PTLDS) or sequelae, and iv) differential diagnosis. A telephone satisfaction questionnaire was conducted one year after the 1st consultation at TBD-RC and consisted in 5 domains and 15 items, including 2 free-text items, rated between 0 (lowest) and 10 (highest grade): (1) Reception; (2) Care and quality of management; (3) Information and explanations given to the patients; (4) Current medical condition (Acceptance of the final diagnosis ; Current condition after the management at the TBD-RC compared to the previous one); (5) Overall appreciation. This questionnaire was validated with patient’s associations. Factors associated with the diagnosis acceptance at 9-12 months were identified using logistic regression models.

Results
Among the 569 patients who consulted, 349 answered the questionnaire (answer rate of 61.5%). There was not any statistical difference of the answer rate between the 4 groups of patients (p=0.44). The overall appreciation had a median of 9 [8;10] and 280/349 (80.2%) accepted their diagnosis. Patients with a proven LB significantly better assessed the TBD-RC than patients with other diagnoses, in all the domains and nearly all the item: they accepted significantly better their diagnosis than patients with a possible LB (p=0.006), PTLDS/sequelae (p=0.001), or other diagnoses (p=0.006); their satisfaction of the final diagnosis and of the global management were significantly better compared to other diagnoses (both p=0.004), and they significantly recommended more the TBD-RC than patients with other diagnoses (p=0.009). Patients “very satisfied” of their care path at TBC-RC (OR=4.64, CI95% [1.52-14.16]), and assessing their current medical condition as “recovery” (OR=2.66, CI95% [0.90-7.88]) had higher odds of diagnosis acceptance. Patients with a possible LB had lower odds of diagnosis acceptance compared to patients with other diagnoses (OR=0.23, CI95% [0.07-0.77]).
Conclusion
Patients seemed to approve this new multidisciplinary care organization for suspected LB. The diagnosis acceptance was associated to the satisfaction of the care paths and the current medical condition of the patients. Patients with a confirmed LB were more satisfied than the other patients, suggesting that these structures are well adapted to LB.
P135 - Factors associated with time-to-treatment in a Mid-Atlantic cohort of patients with erythema migrans

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Introduction
Accurate, timely diagnosis of patients with bacterial tick-borne diseases is important to reduce the risk of infectious complications, however few time-to-treatment studies have been conducted. The aim of this study was to examine whether select clinical and demographic factors are associated with delays in time to treatment in a large sample of well-characterized participants with the erythema migrans rash of early Lyme disease. In a subset of this patient cohort, we also describe the rate and distribution of misdiagnosis.

Methods
Data were drawn from the baseline visit of the longitudinal Study of Lyme Immunology and Clinical Events (SLICE) which is conducted in the Mid-Atlantic region of the US. Adult patients with a visible, diagnostic erythema migrans lesion self-referred or were recruited from primary or urgent care settings. A trained interviewer administered demographic and Lyme disease history questionnaires. Days to appropriate antibiotic treatment initiation was calculated from the participants' self-reported, first Lyme disease sign or symptom. We generated simple linear regression models with days to treatment as the dependent variable and select demographic and clinical factors (including erythema migrans characteristics) as independent variables. Days to treatment was logarithmically transformed, and robust standard errors were applied to all models. Factors with p<0.150 were included in a final multiple linear regression model.

Results
A total of 271 participants were included in the analysis. In a final multiple linear regression model, we found that experiencing viral-like symptoms prior to developing the erythema migrans rash was associated with a 22% increase in time to antibiotic treatment (p=0.012), greater than the effects of other hypothesized factors such as years of education or the classic ‘ring-within-a-ring’ bullseye rash presentation. This patient subset represented a significant minority (38.4%) of our study sample, and first noticed typical viral-like symptoms a median of 3.0 days (IQR: 3.5-10.0, range 0.0-7.0 days) prior to recognition of their rash. Fifteen of 107 (14.0%) who were asked about prior alternative diagnoses were found to have been initially misdiagnosed. While the majority of misdiagnoses were for dermatologic conditions, all non-specific viral misdiagnoses occurred among those who experienced other symptoms prior to erythema migrans.

Conclusions
Absence of the erythema migrans lesion has been previously associated with increases in time to treatment of Lyme disease. This study also highlights the potential significance of those who first experienced viral-like symptoms then subsequently developed or noticed the erythema migrans. These patients may also be at increased risk for early misdiagnosis of other viral-like conditions as a result of anchoring bias, particularly in the era of SARS-CoV-2. Further research is needed to confirm our findings and importantly, to explore how race/ethnicity may impact time-to-treatment, as the distribution of our current sample could not adequately answer that question.
P136 - Post-treatment fatigue and cognitive impairment in Lyme neuroborreliosis patients

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Introduction
Lyme neuroborreliosis (LNB) is generally considered to be the most severe manifestation of Lyme borreliosis. Several previous studies have found that treatment delay is associated with an increased risk of residual symptoms after treatment and is a possible predictor of increased fatigue, reduced cognitive functioning, including processing speed, executive functioning and attention.

Aims
The aim of this study was to determine the prevalence of fatigue and reduced cognitive functioning in LNB patients post-treatment, and to determine whether delayed treatment initiation led to higher levels of fatigue and cognitive impairment.

Methods
The study population consisted of 88 LNB patients included between October 10, 2014 - August 21, 2020 at the Clinical Center for Emerging and Vector-borne Infections at Odense University Hospital, Denmark. The Symbol Digit Modalities Test (SDMT) was used as a cognitive screening test, and the Modified Fatigue Impact Scale (MFIS) was used to assess patients’ level of fatigue over the course of a year.

Results
In all, 14.3% of patients had an SDMT score indicative of cognitive impairment and 38.8% of patients reported experiencing fatigue 12 months post-treatment. We found no statistically significant differences in fatigue or cognitive impairment when comparing the patients who had a treatment delay of ≤14 days and those with a treatment delay >14 days 12 months post-treatment (p.>0.05). However, a random effects regression model showed a significant positive correlation between longer treatment delay and higher MFIS scores, indicating higher levels of fatigue.

Conclusion
The results of this study show that a substantial subgroup of LNB patients still suffer from fatigue and reduced cognitive functioning 12 months post-treatment. They also indicate that patients with shorter treatment delay improve faster than those with a longer delay, although these differences are evened out 12 months post-treatment. We found an association between longer treatment delay and higher levels of fatigue. The study highlights the importance of increased awareness, early detection and early treatment of LNB.
P137 - Cranial Neuropathy versus Bannwarth Syndrome. A Post Hoc Analysis of a Retrospective Cohort Study of Adult Patients with Early Lyme Neuroborreliosis in Europe

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Introduction
Bannwarth syndrome (BWS), a painful meninoradiculitis, sometimes accompanied by peripheral paresis and/or cranial neuropathy is generally considered the dominant manifestation of Lyme neuroborreliosis (LNB) in Europe, but cranial neuropathy with or without meningitis and without radiculitis (CN) also frequently occurs.

Methods
In this post-hoc analysis of data from a retrospective cohort study of early LNB, clinical and microbiologic characteristics and long-term outcome of patients with CN or BWS were evaluated at a single university medical center in Slovenia. Severity of acute disease and long-term outcome during a 12-month follow-up were assessed using a composite clinical score based on objective clinical findings and subjective complaints.

Results
Among 311 adult patients with early LNB diagnosed from 2008 through 2017, the most frequent LNB manifestation was CN (53.4%), followed by BWS (34.1%), meningitis (11.3%), and other neurologic manifestations (1.3%). Patients with CN had less severe disease (median 2 points, interquartile range (IQR) 1–3 points vs. median 4 points, IQR 2.2–5 points), shorter pre-treatment duration of illness (median 11 days, IQR 6–22.5 days vs. median 22 days, IQR 14–45 days), lower rate of accompanying/recent erythema migrans (15.7% vs. 59.4%), and less frequently had a microbiologically confirmed borrelial etiology (37.3% vs. 90.6%) than those with BWS. Unfavorable outcome decreased during follow-up, being higher in patients with more severe disease at enrollment (incidence rate (IR) 1.26, 95% confidence interval (CI) 1.20–1.34), BWS (IR 1.33, 95% CI 1.05–1.67), in women (IR 1.18, 95% CI 1.00–1.32), and in younger patients (IR 1.01, 95% CI 1.00–1.02).

Conclusion
In a highly Lyme borreliosis-endemic European region, early LNB manifested more often as CN than as BWS. The borrelial etiology of BWS may be under-recognized, possibly contributing to a delay in diagnosis and a longer pre-treatment duration of symptoms in BWS than in CN. The higher frequency of accompanying/recent erythema migrans in BWS versus CN could suggest that the borrelial etiology of BWS may sometimes be suspected only when other signs of Lyme borreliosis are recognized.
Patient Expectations and Outcome in Patients with Early Lyme Borreliosis

Introduction
Some patients who receive recommended treatment for early Lyme borreliosis (LB) experience subjective symptoms that may persist after therapy. We aimed to evaluate the association between patient expectations and long-term outcome as assessed by subjective post-LB symptoms in adult European patients with erythema migrans (EM).

Methods
In a prospective clinical trial, adult patients with EM were enrolled and treated in accordance with international guidelines. Patient expectations on treatment outcome were collected at enrolment using a structured questionnaire. Treatment outcome was evaluated at 14 days and at 2, 6, and 12 months after enrolment. Health-related symptoms that had newly developed or worsened since the onset of EM and had no other known medical explanation were regarded as LB-associated symptoms at enrolment or post-LB symptoms at follow-up. Incomplete recovery was defined as the presence of subjective post-LB symptoms.

Results
From May 2018 to May 2020, 298 patients with EM were prospectively enrolled (male 128 [43%], median age 54 years, IQR 43–63).
Median value of patient expectations on efficacy of antibiotics for treating LB (0=ineffective, 10=completely effective) were comparable between patients who reported LB-associated symptoms at enrolment and those who were asymptomatic. The proportion of patients with incomplete recovery represented predominantly by the presence of subjective post-LB symptoms, steadily decreased during follow-up. The multiple logistic regression model for repeated measurements indicated that the probability of incomplete recovery was higher for women and patients with LB-associated symptoms at enrolment. Patients who believed that LB has no post-treatment sequelae did not have significantly lower odds for incomplete recovery (OR 0.59, IQR 0.13–2.68; P=2.68).

Conclusion
Subjective post-LB symptoms were associated with LB-associated symptoms at enrolment and female sex, but not with patients' pre-treatment expectations on treatment outcome. This suggests that interventions to modify patient attitudes towards treatment of LB are unlikely to improve outcomes in LB manifesting as EM.
P139 - Nonspecific Symptoms and Health-Related Quality of Life in Patients with Erythema Migrans

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Introduction
Some patients with early Lyme borreliosis (LB) present with LB-associated symptoms when acutely ill and/or report persistent or intermittent post-LB symptoms after treatment. It is unclear if these symptoms interfere with health-related quality of life (QOL).

Methods
In an open-label clinical trial, performed at the University Medical Centre in Ljubljana, Slovenia, nonspecific symptoms and health-related QOL measured by the RAND 36-Item Health Survey 1.0 (SF-36) were evaluated in adult patients with solitary EM randomly assigned to 7 or 14 days of doxycycline. Symptoms that developed or worsened after initiation of treatment for EM and could not be ascribed to diagnoses distinct from LB were regarded as LB-associated symptoms at enrollment or post-LB symptoms at follow-up. Nonspecific symptoms and health-related QOL were also compared between patients and controls without history of LB.

Results
Among the 300 patients enrolled, 150 (50%) were randomized to receive doxycycline for 7 days and 150 patients were prescribed doxycycline for 14 days. The 2 groups of patients were comparable regarding basic demographic, clinical, and microbiologic characteristics at enrollment. Treatment outcome was also comparable between the two groups. Consequently, both groups were merged for further comparisons. In patients, the frequency of nonspecific symptoms and health-related QOL assessed by an SF-36 questionnaire were comparable at enrollment and at the 12-month visit. Patients and controls did not differ regarding basic demographic parameters. No substantial differences in the frequency of non-specific symptoms or in health-related QOL as assessed by the SF-36 questionnaire were observed between patients and controls at 12 months post-enrollment.

Conclusion
Regardless of treatment regimen, incomplete response was rare and no cases of objective progression of LB were seen. The frequency of nonspecific symptoms and health-related QOL scores in patients at enrollment and 12 months post-treatment as well as in patients and controls without a history of LB were comparable. This suggests that one must remain circumspect when interpreting nonspecific symptoms thought to be associated with LB. Further similar studies should be carried out in other LB-endemic regions to investigate the generalizability of our findings.
Introduction
Lyme borreliosis is the most common tick-borne infection in Bulgaria. Lyme neuroborreliosis (LNB), a manifestation of disseminated Lyme disease, appears with unspecific neurological symptoms. Clinical and laboratory diagnosis are difficult, especially without epidemiological evidence of previous tick bites. Laboratory confirmation of LNB requires determination of antibody index to detect local intrathecal antibody production. Since mid-2018, neuroborreliosis has been included in the updated list, issued from European Commission of communicable diseases to be covered by epidemiological surveillance in the EU.

Aim
To analyse epidemiological features, clinical manifestations and laboratory findings in LNB cases.

Methods
Paired serum/CSF samples from patients (n= 338) with neurological symptoms were tested for specific antibodies against *Borrelia burgdorferi* sensu lato with commercial ELISA (Euroimmun, Germany) in National reference laboratory for vector-borne infections in Bulgaria from 2018 to 2021.

Results: Specific antibodies of IgM and/or IgG class in CSF were detected in 10.9% (37/338) of all tested samples. Both classes IgM and IgG antibodies were observed in 54.0% (20/37) of the positive samples, only IgG antibodies were detected in 29.7% (11/37) and in 16.2% (6/37) only IgM antibodies were found. Distribution of patients by gender shows that affected male were slightly prevalent – 59.4% (22/37). The mean age of the cohort group was 52 years. The most affected age groups were 60-69 years – 29.7% (11/37) and 50-59 years – 18.9% (7/37). Analysis of clinical manifestations revealed polyneuropathy, meningoencephalitis, encephalopathy, paralysis of n.facialis, Guillain-Barré syndrome.

Conclusions
Disease affects patients of all age groups with a tendency for higher morbidity in people in 50th and 60th decade. Simultaneous intrathaelal production of both classes IgG and IgM was detected in more than half of the tested samples. Polyneuropathy, meningoencephalitis and Guillain-Barre syndrome were the most prevalent clinical manifestations of LNB. The patients often underestimate the tick bites and neglect the initial symptoms of infection. This leads to a later diagnosis or in general to misdiagnosis.
P141 - Broad antimicrobial resistance in a case of relapsing babesiosis successfully treated with tafenoquine

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Introduction
Immunocompromised patients are at risk of severe babesiosis, which can be fatal despite antimicrobial therapy. In highly immunocompromised patients, especially those with impaired B-cell activity, prolonged therapy with atovaquone plus azithromycin is recommended but antimicrobial resistance may arise. Anecdotal evidence supports the addition of clindamycin to atovaquone plus azithromycin, although clindamycin and azithromycin both act on ribosomes. An optimized approach to the treatment of babesiosis in immunocompromised patients is needed.

Aims
Tafenoquine, an 8-aminoquinoline approved by the US FDA for malaria prophylaxis and radical cure of Plasmodium vivax infection, has shown activity against Babesia microti in mouse models of babesiosis. We sought to assess the clinical efficacy of tafenoquine against relapsing babesiosis in an immunocompromised patient who presented with clinical and molecular evidence of antimicrobial resistance.

Methods
Babesia microti infection was monitored by examining Giemsa-stained blood smears under light microscopy and/or detecting the Babesia 18S ribosomal RNA gene by real-time PCR. Total DNA was extracted from infected red blood cells and Babesia microti genes suspected of conferring antimicrobial resistance were amplified by use of high-fidelity polymerases. Amplicons were subjected to Sanger sequencing.

Results
An 80-year-old man with a history of cold agglutinin disease was diagnosed with babesiosis on May 20, 2021, four months after initiation of bendamustine and rituximab for monoclonal B-cell lymphocytosis. Initial therapy consisted of atovaquone plus azithromycin but was ineffective. Clindamycin plus quinine were initiated but adverse drug events prompted discontinuation. Recrudescence of low-grade parasitemia was treated with atovaquone plus azithromycin to which clindamycin was subsequently added. A sharp rise in parasitemia justified addition of quinine but gastrointestinal upset caused the patient to interrupt therapy twice. On January 26, 2022, he was admitted to Rhode Island Hospital for relapsing babesiosis. At that time, therapy consisted of atovaquone plus high-dose azithromycin. Parasitemia was 3.1%. A mutation (V141A) in the parasite cytochrome b gene was identified, raising the concern of resistance to atovaquone. A mutation (A1915G) in the parasite 23S ribosomal RNA gene was uncovered, suggesting resistance to both clindamycin and azithromycin. Azithromycin was discontinued and atovaquone-proguanil substituted for atovaquone. Once a deficiency in glucose-6-phosphate dehydrogenase was ruled out, atovaquone-proguanil was interrupted and tafenoquine initiated. A 600-mg loading dose was given over three consecutive days. A 300-mg dose was administered weekly thereafter.
Symptoms quickly resolved; parasites were no longer detected by microscopy or real-time PCR 37 days after initiation of tafenoquine. Tafenoquine was discontinued after a 60-day course. No relapse of symptoms or parasitemia was noted during the 30-day period that followed discontinuation of therapy.

**Conclusion**
Tafenoquine is effective against relapsing babesiosis even when resistance to multiple antimicrobial agents is suspected. Genetic testing for antimicrobial resistance in babesiosis should include the parasite 23S ribosomal RNA gene.
P142 - Doxycycline versus No Antibiotic in Patients with Tick-Borne Encephalitis and Possible Co-Infection with Borrelia Burgdorferi Sensu Lato. A prospective randomized open-label study.

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Introduction
In regions endemic for Lyme borreliosis (LB) and tick-borne encephalitis (TBE), possible borrelial co-infections are reported to occur in 7% to 47% of patients with TBE. It is unresolved whether in such patients, antibiotic therapy may have a favorable effect.

Aims
We aimed to assess whether antibiotic treatment with oral doxycycline, when compared to no antibiotic therapy, was similar in regard to long-term outcome in patients with TBE and possible co-infection with Borrelia burgdorferi sensu lato.

Methods
We enrolled adult patients with TBE and possible borrelial co-infection defined as presence of borrelial IgM or IgG antibodies in serum or cerebrospinal fluid (CSF) in an open-label randomized clinical trial, conducted at the University Medical Center Ljubljana between July 2014 and September 2020. Patients with TBE and clinical or microbiologic evidence of proven borrelial co-infection, defined as presence of recent or concomitant erythema migrans and/or intrathecal synthesis of borrelial IgM or IgG antibodies and/or isolation of borreliae from CSF, were excluded. Patients who received antibiotic treatment with antiborrelial activity for other indications in the course of TBE were also excluded. Patients were randomized 1:1 to receive oral doxycycline 100 mg bid for 14 days or no antibiotic therapy. We assessed antibiotic efficacy based on clinical and microbiologic parameters (development of objective clinical manifestations of LB and/or seroconversion and/or ≥ fourfold increase of borrelial serum antibodies) at follow-up at 2, 6 and 12 months after enrollment.

Results
Out of 78 enrolled patients, 40 patients were treated with doxycycline and 38 did not receive antibiotic therapy. At the last evaluable follow-up visit, none of patients in both groups developed an objective clinical manifestation of LB. At least fourfold increase of borrelial serum IgG antibodies was established during follow-up in two patients from the doxycycline group (2/40, 5.0%; 95% confidence interval (CI) 0.6% to 16.9%) and in none from the group without therapy (0/38; 95% CI 0% to 9.3%); risk difference was 5.0%; 95% CI -5.2% to 16.9%.

Conclusion
In patients with TBE and possible borrelial co-infection, long-term outcome in regard to developing clinical and/or microbiologic manifestations of LB was not affected by treatment with doxycycline. This suggests that in patients with TBE and possible borrelial co-infection, antibiotic therapy may safely be deferred.
P143 - Determinants for persistent symptoms after treatment for Lyme borreliosis: predictive and associated factors in a prospective cohort in the Netherlands

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Introduction
Little is known about risk factors for, or pathogenesis of, persistent symptoms after treatment for Lyme borreliosis (LB). Previously, we reported a 4 to 6% higher prevalence of long-lasting symptoms in patients treated for LB than in reference cohorts of individuals without LB, with a substantial background prevalence of more than 20%. The current study describes the exploratively assessment of a broad range of pre-defined potential determinants for these persistent symptoms in the large prospective LymeProspect cohort.

Methods
In a cohort of 1135 physician-confirmed LB patients we measured microbiological, immunological, genetic, clinical, epidemiological, and cognitive-behavioral parameters as potential determinants for persistent symptoms. Data were obtained through questionnaire and laboratory measures at the start of antibiotic treatment and during one year of follow-up. Determinants for persistent symptoms were identified using association studies and random forest prediction analysis. For comparison, a subset of longitudinal questionnaire variables was available for two reference cohorts of individuals without LB.

Results
Poorer social and physical functioning, higher IPQ scores (poorer illness perception), and higher HADS anxiety and depression scores at start of antibiotic treatment were identified as determinants for persistent symptoms. These variables contributed to a 72% correct prediction of persistent symptoms by random forest analysis. Laboratory measurements, such as B. burgdorferi s.l. serology, SNPs related to the host-immune response against B. burgdorferi s.l., and cytokine production upon stimulation of immune cells with B. burgdorferi s.l. had at most limited predictive value for persistent symptoms. Likewise, clinical characteristics related to the infection, such as duration of symptoms before treatment and EM size, did not relevantly contribute. In a subgroup analysis of LB patients with persistent symptoms who themselves attributed their persistent symptoms to LB, laboratory and clinical variables related to disseminated LB and longer antibiotic treatment courses were the most important predictors for self-attribution of symptoms to LB. In joint analyses of the LymeProspect cohort and both reference cohorts, confirmed LB itself was significantly associated with persistent symptoms, but only moderately important in multivariate prediction analysis.

Conclusion
In the primary analysis, determinants for persistent symptoms after LB in this study were mainly generic, whereas disease-related factors did not add substantial predictive value. This might well be explained by the substantial background prevalence of these symptoms, indicating that
persistent symptoms are not related to *B. burgdorferi* infection in a large part of the LB cohort. Subgroup analysis showed that laboratory and clinical determinants related to more severe acute infections are predictive of attributing persistent symptoms to LB. Irrespective of pathophysiology, persistent symptoms after LB were clearly associated with impaired physical and mental quality of life.
P144 - Don’t let a tick make you sick! Content analysis of French resources on Tick bite prevention available in France

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Introduction
Lyme borreliosis (LB) is the most frequently transmitted infection following a tick bite in France. Its incidence doubled since 2009 and stood at 60083 cases/year by 2020. The application of preventive measures against tick bites during outdoor activities is the best way to avoid contracting it. A study published at the end of 2021 by Santé Publique France showed a high perception of the risk related to tick bites (74%) in the general population. However, the level of knowledge and application of prevention measures was lower among young people and men living in low-endemic areas. To date, the consistency of information about tick bite prevention on French websites d has not been evaluated.

Aims
We aim to compile a list of available resources accessible on the Internet by the general population on tick bite prevention. We then analysed the types of information materials available and their content in order to identify the current limitations of the communication strategy on Lyme disease prevention in France.

Methods
Inventory of information resources available online on tick bite prevention from institutional actors, patient associations, conventional and social media. We then characterised and reviewed the content of these materials using a socio-anthropological approach in order to establish their relevance to the target populations.

Results
Information on tick bite prevention was mainly available on the websites of institutional actors and patient associations. The conventional and social media mainly communicated on lyme borreliosis but little on its prevention. Search time was shorter and the information more concentrated on the websites of patient associations, which contained information sheets aimed at targeted audiences (people with a garden, outdoors activities, professionals exposed to the disease, pet owners). The main information material used were similar: web pages, PDFs, flyers, posters, podcasts, videos, and educational materials for children. Analysis of the content of these information material revealed major differences in terms of computer graphics and semantics. The information relayed by the associations was expressed in more simplified language but also more alarmist than that of the institutional actors. They notably sed more videos depicting real life exposure situations.

Conclusion
A wide variety of information media are available online to the general public. This multiplicity can be a source of confusion and lead to a dilution of the message. It would be necessary to build a common communication strategy to provide accessible, evidence-based information to the public, including all the identified actors. The language and tools must be adapted to the target
population, in order to reinforce the effectiveness of this prevention campaign, especially among men and young adults living in low endemic areas but likely to travel to high endemic areas especially during their holidays.
Targeted approaches to study the interplay between pathogens and their hosts have used approaches that include the analysis of mutants and host immunological responses. Although these studies do provide a focus on individual pathways they fail to reveal the global effects of infection on the host. Hence to overcome such barriers, high-throughput methods, such as transcriptomics and proteomics, have been used to study host-pathogen interactions. Over the last decade metabolomics has been established as the method to study the biochemical composition of host tissues. Here we report on a metabolomics study of *Borrelia afzelii* infection in a murine model upon natural tick transmission of the bacterium. This work was carried out to help complement proteomic data of various organs in infected animals when fed upon by infected ticks that were maintained at two different temperatures. Ticks infected as larvae were allowed to feed on naïve C3H mice to repletion. 3 weeks post feeding the mice were assessed by Western Blots for infection by *B. afzelii*. Focusing on the heart, liver, spleen and thymus from both infected male and female mice, our results revealed that dozens of host metabolic pathways are affected by Borrelia infection and is affected in opposite manner when the host is infected with Borrelia transmitted from ticks at different temperature. In particular, multiple pathways including glycolysis are disrupted. Our results identify unappreciated effects of infection on host metabolism. Information based on the up-regulation and down-regulation of certain reactions could be utilized to draw new treatment strategies against Lyme infections.
P145 - Tick bite prevention in high-risk populations: current practices and the evaluation of tick training tools at summer camps in Wisconsin, United States.

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Introduction
Children, 5 to 14 year-olds, have one of the highest Lyme disease incidences in the United States. This age-group also represents a large group of summer camp attendees. Summer camps host over 14 million campers annually in the United States. These camps coincide temporally with peak tick activity and campers engage in activities within tick habitat, possibly increasing their chances of infectious tick bites.

Aims
To reduce tick-borne disease risk at camps we 1) assessed current practices and needs from camps in a high Lyme disease endemic area, and 2) compared the effectiveness of two tick training tools, resin-embedded ticks and the CDC-tick drawings, as education tools.

Methods
In 2019, staff was trained to give campers tick training at a camp in South Central Wisconsin, and 98 Wisconsin summer camps were invited by mail to participate in a 5 to 10-minute online survey. The survey consisted of four sections: camp characteristics, tick season, intervention, and barriers/willingness to implement interventions.

Results
Campers were reportedly bitten by ticks at least occasionally at half of the camps (15 out of 29). The majority of camps used camp staff as educators for campers (20 of 21), but only 57% of camps taught their staff about tick recognition, ticks/Lyme disease risk, prevention of tick bites and removal of ticks. Many camps taught campers to do tick checks (22 of 30), but it is unknown how well campers know what ticks look like. All camps surveyed were not fully satisfied with their current tick prevention strategies and the main concerns in pursuing additional measures were time and cost required.

At one camp, a total of 149 tick training sessions with 1047 participants were completed. The type of tool did not change the average length of the training, 5-7 minutes, but counselors reported increased engagement of campers with the resin-embedded ticks compared to the tick-illustrations. In addition, the campers who experienced the resin block training tended to be more likely to find a tick on themselves, 13.3% versus 10% respectively.

Conclusions
Summer camps provide a high-risk environment for tick exposure and are willing to implement training, but budget and time are limiting. Access to easy to implement and cost-effective strategies to improve tick-borne disease prevention for camp staff and campers could increase the quantity and quality of prevention strategies used at camps. The implemented staff-led tick training was well received, and resin-embedded ticks may be a more effective tool to improve tick checks compared to an illustration of ticks. These findings warrant additional research to evaluate if these results are generalizable to other camps and possibly could reduce Lyme disease cases in children after camp participation.
P146 - Poor recognition of the vector of Lyme disease in resin-embedded specimens in a Lyme endemic area, Wisconsin, USA

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Introduction
Lyme disease (LD) is the most common vector-borne disease in the United States. In the eastern United States only one of the human biting ticks transmits \textit{Borrelia burgdorferi} s.l.. To assess whether a tick bite puts an individual or patient at risk for LD, adequate tick identification skills are needed. However, tick identification was moderate among 76 Northeastern USA healthcare providers and had not been assessed among the public.

Aim
We surveyed residents of a high LD-incidence state, Wisconsin, on their ability to distinguish ticks from insects and to identify the specimens that could transmit the LD causative agent.

Methods
Surveys were conducted using resin blocks with four insects and four tick specimens embedded: a flea, an Eastern ash bark beetle, a swallow bug, a drugstore beetle, an \textit{Amblyomma americanum} adult, \textit{Dermacentor variabilis} female, \textit{Ixodes scapularis} nymph and adult female. Specimens were preserved in resin in small Petri dishes. Surveys were carried out orally by Midwest Center of Excellence for Vector-borne Diseases team members. Responses were registered in Qualtrics. Participants were recruited opportunistically. The survey consisted of four sections: 1) obtaining consent; 2) demographic information; 3) assessment of agreement with three statements: I know a lot about ticks, I am very worried about Lyme disease, I am very outdoorsy; 4) tick and Lyme disease vector identification.

Results
About half of the participants (64 of 130) recognized all of the ticks, and 60\% of those individuals chose only ticks and no insects. Younger participants (18- to 44-yr old) were more likely to identify ticks correctly compared with those 45 yr and older. Participants who agreed strongly with the statement ‘I know a lot about ticks’ were also more likely to correctly identify ticks. When asked to identify which specimens could transmit LD, less than 25\% of participants chose both the \textit{Ixodes scapularis} adult female and nymph and about half of those (15\% of participants) picked only those two and no other specimens.

Conclusion
Although the relatively small convenience sample was biased toward younger participants who consider themselves ‘outdoorsy’, results showed that further assessments of tick recognition skills are needed to understand what determines whether people can recognize medically important ticks and to evaluate the potential benefits of enhanced education. In addition to the value of the resin blocks as research tools, the blocks may be useful as training tools to improve tick check efficacy.
P147 - Addressing Lyme Disease via Reservoir Targeted Antibiotic

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Introduction
Hygromycin A (HygA) has recently been shown to be active against Borrelia burgdorferi. A major advantage of HygA is that it has a very narrow spectrum of activity that is largely limited to spirochetes. Narrow spectrum antibiotics are less likely to engender resistance in non-target bacteria. This aspect of HygA may have particular appeal for its use as a reservoir targeted antibiotic given to mice to eradicate B. burgdorferi in the wild. Treatment of mice with another antibiotic, doxycycline, has been shown to be highly effective in eradicating Borrelia burgdorferi from ticks and mice in the wild. However, there is legitimate concern for development of resistance, both in B. burgdorferi and in other organisms that may be exposed to the antibiotic should it be widely distributed. We are testing HygA as a potential reservoir targeted antibiotic for mice.

Methods
Peromyscus mice were infected with B. burgdorferi by tick or needle inoculation and then administered doses of HygA by oral gavage. Ear punch cultures were obtained to confirm infection prior to treatment, and to monitor infections during treatment. Mice were then sacrificed at the end of each study, and organs were cultured in BSK-II to determine clearance of infection. We tested a single high dose of 1,500 mg/kg by oral gavage as well as by bait.

To test the efficacy of HygA under more natural feeding conditions, we created HygA containing baits that were placed in cages with four B. burgdorferi infected mice each. The amount of HygA was sufficient to provide a dose of 1,500 mg/kg/mouse if each mouse ate the bait equally. Mice ate the baits quickly and 100% of the baits were eaten in all cages by 24 hrs. We were not able to track the amount of HygA ingested by each mouse. Mice were euthanized and checked for infection 2 weeks after consuming the bait.

Results
We tested a single high dose of 1,500 mg/kg by oral gavage as well as by bait. 100% of mice were cleared by day 14 when treated by oral gavage. 66% of the mice in the bait trial were cleared of infection after 14 days. Each of the four mice that remained positive had a singular tissue culture with borrelia growth.

Conclusion
HygA is an effective antibiotic for the eradication of B. burgdorferi from Peromyscus mice. Even after a single exposure to HygA, mice were cleared of B. burgdorferi infection. If used in the wild, mice would likely have more than a single exposure to HygA, resulting in even higher clearance rates. HygA is an attractive candidate for use as a reservoir targeted vaccine due to its narrow spectrum and its high efficacy against B. burgdorferi infection.
P148 - Analysis of tick regurgitation following mechanical, cryogenic and chemical removal

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Introduction
Common advice from health authorities is to remove a tick using tweezers or a tick removal device. Squeezing of the tick should be avoided as it may induce regurgitation and thereby increase the risk on allergic reactions or pathogen transmission. There are few studies in the scientific literature that have systematically compared tick removal methods in relation to the risk of exposure to tick-derived allergens or pathogens. Here we describe a bioassay that analyses tick regurgitation following mechanical, cryogenic and chemical removal.

Methods
First, we optimized membrane feeding of Ixodes scapularis. We subsequently marked the midgut contents by feeding on blood containing fluorescein dye. Following transfer to a clean feeder, reattachment, and placement of the feeder on 0.9% saline, ticks were removed by different methods and release of fluorescein into the liquid compartment was analysed as a measure for removal-induced regurgitation. We compared mechanical removal by household tweezers to rapid freezing of ticks using dimethylether in a spray cannister. In addition, and with a view on development of systemic acaracides for human use, we analysed regurgitation following chemical removal using fipronil as a tool compound.

Results
Initial attachment and feeding rates on silicon membranes were 73 and 60% for nymph and adult stages, respectively. Fluorescein in the bloodmeal was well tolerated up to concentrations of 5 mM. Partially-engorged ticks were transferred to clean feeders and reattachment rates were between 33 and 83%. Following placement of feeders on 0.9% saline solution, all feeders caused some background accumulation of fluorescein in the lower compartment, possibly due small leaks causing contact between the saline solution and tick excrements. Following removal of ticks using household tweezers, fluorescence intensity strongly increased in 7 out of 10 feeders. This was not observed in 10 feeders where ticks were removed by the cryogenic method.

Conclusion
Our data indicate a higher release of fluorescein upon mechanical removal in comparison with cryogenic removal. This suggests a higher risk on regurgitation, although we cannot fully exclude that the mechanical manipulation leads to release of fluorescein from other sources than the tick midgut or saliva. Studies on chemical removal are ongoing. We will discuss the results of the fluorescein measurements, potential implication for risk on tick-induced diseases and future directions of these studies.
P149 - Rationale for Taking a Total Worker Health Approach to Tick-borne Disease Prevention

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Introduction
Employees in high-risk occupations and working in tick endemic areas need to be protected from the workplace hazard of ticks and have access to education and resources for preventing tick-borne diseases (TBDs). There is currently a lack of attention to TBDs by many occupational safety and health (OSH) organizations and employers, most notably the United States Occupational Safety and Health Administration who has no known national standards. As tick-borne diseases continue to increase and expand geographically, an evidence-based rationale and approach for employers and OSH organizations to include tick-borne disease prevention as part of their workplace health, safety and well-being programs is urgently needed.

Methods
A mixed method survey was conducted with 434 adults diagnosed with one of 18 tick-borne diseases in 2021, and included open ended questions related to impacts on work life. Snowball sampling was used to recruit participants through email and social media sites. Qualitative responses were hand-coded and qualitative data analysis software was used to explore patterns, frequencies and themes. A literature review of evidence-based OSH approaches and workplace tick-borne disease education and prevention programs was also conducted.

Results
Three in five (60.3%) participants reported their tick-borne disease symptoms interfered with their ability to work. Symptoms affected participant’s ability to go to work (absenteeism), be fully present while at work (presenteeism) or be able to perform their job responsibilities at their highest potential (productivity). In some cases, employees were fired, forced to quit or had to switch to a different profession because of the severity of their symptoms (all TBDs) or because of allergic reactions resulting from workplace exposures (Alpha-gal Syndrome). While a few participants reported being exposed while working, of those who knew where they were exposed (N=272), one in two (50.4%) were exposed in their yard followed by at a park or while recreating (25.4%). Findings from the literature review suggest that applying the Health Belief Model to tick-borne disease prevention can protect workers by changing personal protective behaviors, and that a taking Total Worker Health approach can not only protect workers, but also prevent illness and advance worker well-being.

Conclusion
Symptoms associated with tick-borne diseases can significantly impact an employee’s ability to work, thus affecting an employer’s bottom line through decreased productivity and increased absenteeism, presenteeism and healthcare costs. Employers and occupational safety and health organizations need to recognize the effects that having a tick-borne disease can have on employees and understand that exposure to ticks can occur both on and off the job. A Total Worker Health approach to workplace tick-borne disease prevention that applies the HealthBelief Model is recommended as an evidenced-based strategy for tick-borne disease prevention.
P150 - Barriers to uptake of tickborne disease prevention measures: Connecticut and Maryland, USA

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Introduction
Tickborne diseases (TBDs) are increasing in the United States, with the largest burden due to Lyme disease. Despite their ability to reduce tick encounters and TBDs, prevention measures are not always consistently performed. We examined barriers to performing TBD prevention measures among residents in states with high incidence of Lyme disease.

Methods
During 2016-2017, we conducted a survey to evaluate knowledge, attitudes, and behaviors related to TBD prevention among 1883 persons living in areas endemic for Lyme disease in Connecticut and Maryland, USA. In a previous analysis, frequency of performing tick checks, applying insect repellents to oneself, showering/bathing 2-hours after outdoor activity in tick habitat, treating pets against ticks, willingness to use permethrin-treated clothing, and applying chemical or natural pesticides to residential lawns were assessed. Respondents could choose multiple reasons for never, rarely, or sometimes performing these measures. In this study, we identified the primary barriers to performing these common TBD prevention measures.

Results
The three most cited barriers to each TBD prevention measure were as follows: For tick checks (n=800), 55% forgot, 25% reported not spending time in tick habitat, and 9% said it was too much trouble. For applying insect repellents to oneself (n=1303), 28% forgot, 18% had personal safety concerns, and 14% said it was too much trouble. For showering/bathing 2-hours after outdoor activity in tick habitat (n=1080), 42% did not know this prevented TBDs, 15% said it was too much trouble, and 15% forgot. For treating pets against ticks (n= 183), 32% said pets don’t spend time in tick habitat, 16% had pet safety concerns, and 16% had other reasons. For willingness to use permethrin-treated clothing (n=330), 35% had personal safety concerns, 13% had cost concerns, and 11% were not concerned about TBDs. For applying chemical pesticides to lawns (n=1320), 26% had environmental concerns, 18% had pet safety concerns, and 16% had personal safety concerns. Lastly, for applying natural pesticides to lawns (n=1357), 23% did not know about natural pesticides, 17% had cost concerns, and 12% were not concerned about ticks on their property.

Conclusion
Forgetting to perform and unfamiliarity with certain types of TBD prevention measures were the main reasons for respondents not performing them. This highlights the need for passive tick control methods, such as a Lyme disease vaccine, to provide protection when people forget to use personal prevention measures. Additionally, education regarding the effectiveness of certain measures (bathing/showering 2-hours after outdoor activity in tick habitat, natural pesticides), safety (applying insect repellent to oneself, treating pets, permethrin treated clothing, chemical pesticides), and seasonal timing for use of measures is warranted to increase uptake of available measures to reduce cases of TBDs.
Control and prevention strategies for tick-borne diseases require knowledge about where and when people get bitten. However, little is known about where in Denmark people get tick bites. Additionally, the public needs reliable information on the prevention and symptoms of various tick-borne diseases.

A Danish tick website was launched in August 2021 and was advertised in various Danish media e.g. websites and magazines to increase awareness. It is a public webpage where people can register tick bites on humans and pets, crawling ticks, and rashes. By also encouraging registrations from pets, we can use these as indicators of human risk. Age and gender are also reported along with each registration. Lastly, it is possible to upload pictures of ticks and rashes.

By the end of March, we have received a total of 540 registrations and 72 pictures of rashes and ticks. Most registrations are from humans (n=366) compared to pets (n=153).

Although the website has only been available from late summer to early spring, 540 registrations outside tick season show that people have an interest in ticks outside peak season and that bites occur throughout the year. Covering the peak tick season in the coming years will bring more registrations and more awareness about ticks and tick-borne diseases. This year we will also encourage people to send the ticks to us.
Poster pitches

PP01 - Duration of symptoms among clinician-diagnosed Lyme disease patients in the Northeast and Upper Midwest, United States

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Epidemiology, September 5, 2022, 10:50 AM - 12:30 PM

Introduction
There are nearly 500,000 diagnosed cases of Lyme disease (LD) in the United States annually, yet information on duration of illness after acute infection is limited. As part of a cost of illness study, we prospectively collected symptom and healthcare utilization data from LD patients.

Methods:
Clinician-diagnosed pediatric and adult LD patients were recruited through public health surveillance in Connecticut, Maryland, Minnesota, and New York and met the U.S. surveillance case definition for confirmed or probable LD. Recruitment was stratified by disease category (confirmed early, confirmed disseminated, and probable disease), and data were weighted according to disease category distributions from national surveillance data. Participants were surveyed approximately monthly, for up to 12 surveys, to collect symptom onset date, diagnosis date, type and frequency of healthcare-related visits, and type and duration of symptoms. Descriptive statistics were performed. As the primary objective of the study was to evaluate the economic burden of LD, data collection ended when costs ceased for the participant or after 12 surveys.

Results
We enrolled 901 individuals. Most were male (57%) with a median age of 49 years; 14% had reported a prior LD diagnosis. Median days from symptom onset to diagnosis was 16 (mean: 30, range: 0-323). Median days from the first healthcare visit to diagnosis was 4 (mean: 12, range: 0-304). Median days from symptom onset to resolution was 102 (mean: 137, range: 10-664). Those with confirmed disseminated LD consistently had the longest duration for the above intervals, followed by those with probable then confirmed early LD. Participants reported a median of 2 healthcare provider visits (mean: 3, range: 1-47), 1 laboratory visit (mean: 1, range: 0-34), and 2 pharmacy visits (mean: 2, range: 0-92).

The percentage of participants reporting any symptom ranged from 98% at enrollment to 4% at the end of study participation. At illness onset, most participants reported fatigue (71%), joint issues (60%), EM rash (57%), muscle pain (55%), fever (49%), and headache (48%). At the end of participation, the most common symptoms reported were fatigue (3%), joint issues (3%), muscle pain (2%), stiff neck (2%), headache (1%), chills/sweats (1%), and other symptoms (1%). Participants reported a median of 5 different symptoms during study participation (mean: 5, range: 0-13).

Conclusions
Presence of symptoms decreased over time. Few participants reported any symptom past 5 months after diagnosis, and it was rare for symptoms to persist at 12 months. Potential limitations of this study include recall error in self-reported symptoms and dates. Given censorship at approximately 12 months post-enrollment, duration of illness for a small percentage of individuals could not be ascertained. Early and accurate diagnosis and treatment, along with improved prevention methods (e.g., vaccines), are needed to minimize the potential for persistent symptoms.
**PP02 - Persistent Borrelia burgdorferi sensu lato infection after antibiotic treatment: a systematic overview and appraisal of the current evidence from experimental animal models**

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Epidemiology, September 5, 2022, 10:50 AM - 12:30 PM

**Introduction**

Lyme borreliosis (LB), caused by *Borrelia burgdorferi* sensu lato (*Bb*sl) spirochetes, induces diverse clinical manifestations that are treated with antibiotics. In some patients, long-lasting and debilitating symptoms can persist after recommended antibiotic treatment, often referred to as post-treatment Lyme disease syndrome (PTLDS). A highly debated hypothesis regarding these persisting symptoms entails the existence of a persisting infection by a subset of spirochetes surviving antibiotic treatment, so-called *Borrelia* persistenters. This review entails all causes of persisting infection after antibiotic treatment, including host-factors (the immune system), treatment-factors (adequate treatment) and pathogen-factors (including resistance, tolerance and/or persistence). Evidence from experimental animal studies on this topic is described here.

**Material and methods**

The PubMed database was searched for studies on persistent *Bb*sl infection after systemic antibiotic treatment in mice, dogs and non-human primates (NHP). Searches were performed and data were extracted, appraised and reported per animal model by two researchers. The primary outcome was culture positivity. Secondary outcome measures were: molecular diagnostics, xenodiagnosis, immunofluorescence assay, direct fluorescence assay, immunohistochemistry, serology, histopathology, joint measurements and allografting. Exclusion criteria were: insufficient data to determine persisting infection (only one secondary outcome examined), wrong publication type, language other than English or no-full-text availability. The review focusses on most clinically used antibiotics doxycycline and ceftriaxone, while data for all antibiotics were distracted.

**Results**

A total of 30 studies were included for the murine-, 5 for the canine- and 5 for the NHP model. Doxycycline and ceftriaxone were highly effective in clearing a *Bb*sl infection in majority of studies. Our review indicates that some *in vivo* animal studies found sporadic positive cultures after antibiotic treatment. However, this culture positivity oftentimes seemed to be related to inadequate treatment or immunocompromised status of the animal. *In vitro* examination of bacterial factors contributing to persistent infection after antibiotic treatment, such as minimal inhibitory concentration determination, was only one time performed in a positive culture from an
immunocompromised mouse. The sporadic positive cultures could not be reproduced in other studies. On the contrary, low levels of borrelial DNA could be detected by PCR following various antibiotic regimens in all animal models.

Discussion/conclusions
Although borrelial nucleic acids may persist after antibiotic treatment, persistence of viable and infectious spirochetes remains unproven based on current published animal data. There is insufficient evidence to conclude that persisters play a role in persisting \textit{Bb}sl infection after adequate antibiotic treatment, let alone an infection explaining PTLDS. Research into the pathogenesis of PTLDS should remain a top priority, but the focus of such research should also encompass other explanations than a persisting \textit{Bb}sl infection.
PP03 - Transcriptome mapping of B. burgdorferi reveals RNA regulators

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Epidemiology, September 5, 2022, 10:50 AM - 12:30 PM

Introduction
B. burgdorferi, the causative agent of Lyme disease, has a limited number of characterized transcription factors, despite existing in a complex enzootic cycle involving Ixodes scapularis ticks and mammals. RNA-based regulation is an attractive mechanism for fine-tuning gene expression during the transmission and survival of these tick-mammalian environments. To date, few examples of small base-pairing RNAs (sRNAs) and no examples of cis-acting RNA elements (such as riboswitches, RNA thermometers and attenuators) have been reported.

Methods
To discover novel RNA regulators, we combined three RNA-seq approaches to globally map B. burgdorferi 5’ and 3’ RNA boundaries.

Results
This resulted in a list of 217 promising sRNA candidates, 44% of which were novel to this study. We confirmed expression of 37 sRNAs by northern analysis, revealing unique patterns in levels and processing across bacteriological growth. The transcriptional activity of 10 sRNAs, including several encoded proximal to genes for infection-relevant proteins, was measured with promoter-luciferase fusions during an active mouse infection model of Lyme disease. The transcription of some sRNAs was specifically induced during mammalian infection compared to other sRNAs. The base-pairing and regulatory effects of one sRNA was further characterized, revealing the novel sRNA alters B. burgdorferi growth and cell length. This sRNA was further shown to affect the level of mammalian-specific RNAs and the ability of spirochetes to efficiently establish a mammalian infection. Simultaneously, we used our RNA-seq data to improve B. burgdorferi gene annotations, map mRNA untranslated regions (UTRs) and characterize transcription termination. We discovered numerous instances of premature transcription termination, a hallmark of cis-acting RNAs. In one example, a 5’ RNA fragment corresponded to the 5’ UTR of a spermidine importer, potB, an essential gene. The levels of the 5’ transcript increased with the addition of spermidine with a concomitant decrease in the downstream potB mRNA. Promoter and translational fusions to luciferase document that this affect is dependent on the potB 5’ UTR. Therefore, we hypothesize this represents a cis-acting regulatory RNA.

Conclusion
Collectively, this work maps RNA boundaries of the B. burgdorferi transcriptome and identifies novel RNA regulators important during infection.
PP04 - Investigating small and medium-sized mammals to identify potential reservoirs of Borrelia miyamotoi in the North Central U.S.A.

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Epidemiology, September 5, 2022, 10:50 AM - 12:30 PM

Introduction
The causative agent of Borrelia miyamotoi disease was discovered over two decades ago, but its enzootic cycle remains undefined. In the eastern United States, B. miyamotoi is vectored by Ixodes scapularis and has been detected from several small mammals whose reservoir status is unknown. Identifying potential reservoir host species and estimating their relative importance for enzootic maintenance will improve our understanding of the ecology of this vertically-transmitted pathogen and direct future research efforts.

Methods
For three years, we live-trapped small and medium-sized mammals in a forest in the north central U.S.A. where I. scapularis is abundant. We collected blood, ear tissue, and on-host ticks. We screened the collected samples for B. miyamotoi infection by PCR.

Results: From a total of 2152 captures of 14 mammal species, the white-footed mouse (Peromyscus leucopus) was the most frequently trapped species and hosted the majority of collected larval (72.2%) and nymphal (50.7%) I. scapularis. We observed the highest mean larval load on white-footed mice (5.1 ticks/capture) and the highest mean nymphal load on eastern chipmunks (Tamias striatus; 2.6 ticks/capture). A total of 21 individuals comprising five species were identified as infected with B. miyamotoi; 13 of the infected individuals were white-footed mice, but the eastern chipmunk showed the highest infection prevalence (3/16; 18.8%).

Conclusions
Based on their relative abundance, contact with vectors, and infection prevalence, both the white-footed mouse and eastern chipmunk may have greater ‘reservoir potential’ for B. miyamotoi compared with other species we captured. Based on field estimates of infection susceptibility, infection persistence, and transmission efficiency, we infer that the white-footed mouse may have lower reservoir competence for B. miyamotoi than the eastern chipmunk. Controlled laboratory studies (e.g., xenodiagnostic experiments) are needed to further clarify the importance of these species for B. miyamotoi enzootic maintenance. Overall, B. miyamotoi infection prevalence among small mammals, however, was low, and may suggest a role for other host species we did not capture. Modeling efforts may help elucidate the relative importance of vertical and horizontal transmission and guide future research efforts in the field and laboratory...
Introduction
Correct diagnosis of Lyme disease (LD) still represents a big challenge worldwide. LD is caused by spirochetes of the *Borrelia burgdorferi* sensu lato (*Bb*sl) complex and is transmitted to humans by the bite of infected ticks. In North America, *Borrelia burgdorferi* sensu stricto (*Bb*ss) is the dominant genospecies associated with human LD; in Eurasia *B. afzelii* and *B. garinii*, are the two most prevalent pathogenic species. Scientific evidence suggests that different *Bb*sl genospecies differ in terms of expressed antigens, disease presentations, response to antibiotics and different clinical manifestations have also been recognized due to a different organotropism. This introduces diagnostic issues and poses additional question marks about the optimal management of patients. Here, we describe a multiplex peptide ELISA to allow serological genospecies identification of the three key *Bb*sl species.

Materials and Methods
Diagnostic peptide epitopes were chosen through (a) systematic review of outer surface proteins (Osp) of immune-diagnostic value, (b) selection of proteins with evidence of ortholog sequence divergence and (c) B cell epitope prediction and modelling. Genospecies ortholog specific epitopes from 7 oligopeptide regions in 5 Osp, were finally synthesised and evaluated in a multiplex peptide ELISA testing a panel of US and EU early-stage and late-stage Lyme disease sera.

Results
The 7 oligopeptide panel achieved the desired sensitivity and accuracy. Sensitivity and correct genospecies identification were 56.2% and 88.8% respectively for IgM responses, and 71.8% and 95.6% respectively for IgG, responses. Higher diagnostic power was observed when IgM and IgG antibody detection was combined. This gave sensitivity and correct genospecies identification of 90.6% and 96.5%, respectively.

Discussion
The multiplex ELISA platform is highly effective in discrimination of infections with *Bb*ss, *B. garinii* or *B. afzelii*. In the longer term, diagnostic peptides could be incorporated into a point of care rapid test.
Designing a cocktail vaccine against Lyme borreliosis combining tick- and Borrelia-derived antigens

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Introduction
Lyme borreliosis (LB) is the most common vector-borne disease in the Northern hemisphere and causes a spectrum of clinical manifestations, including arthritis, cardiac issues, and neurological abnormalities. The steadily climbing incidence and lack of a human vaccine demands the development of new, potent vaccines.

Aims
We have identified and evaluated novel vaccine candidates for LB, including proteins from both the vector (Ixodes ticks) and the pathogen (Borrelia burgdorferi sensu lato). This novel approach represents a dual-edged sword that could provide protection against not only LB, but also other tick-borne diseases (TBDs).

Methods
Over 200 pathogen-derived vaccinogens were identified using a protein microarray, which examined immune responses to approximately 1300 Borrelia afzelii proteins using sera from humans (n=149) and mice (n=48) during different disease/infection stages. Three early stage and highly immunogenic Borrelia antigens (Baf1-3) were selected for evaluation as candidate vaccines. Mice were immunized with a combination of 10μg of recombinant, unlipidated Baf1-3 in alum and subsequently challenged with B. afzelii-infected I. ricinus nymphs.

In the second arm of the approach, 124 salivary gland antigens from I. ricinus were identified as highly up-regulated and abundant within 24 hours of tick feeding using Massive Analysis of c-DNA Ends and RNA sequencing. Twenty candidates were further validated by biological verification in independent, uninfected tick pools. Selected candidates were characterized by in vitro functional assays, and one tick protein (TSGP1) was implicated in promoting Borrelia transmission and survival. Based on these results, mice were vaccinated with 20μg recombinant TSGP1 in Complete/Incomplete Freund’s Adjuvant and infested with B. afzelii-infected I. ricinus ticks.

Results
Active vaccination with Baf1-3 induced strong IgG responses (mean endpoint titers $10^5$-$10^6$), but did not provide significant protection against B. afzelii challenge. This result could be attributed to the antigens’ lack of lipidation, which could prevent native folding and formation of functional antibodies against these lipoproteins. In future studies, we intend to optimize efficacy by immunizing with a higher dose of lipidated Baf1-3 and by modifying inoculum dose and method. TSGP1 vaccination was highly immunogenic and yielded partial but significant protection against challenge with B. afzelii-infected ticks in two studies, substantially reducing spirochete load in all tissues compared to unvaccinated mice. Additional in vitro functional data implied two other
tick proteins (TSGP2 and 3) shield *Borrelia* from complement-mediated killing, supporting their potential as pathogen-blocking vaccine candidates.

**Conclusion**
We have identified and evaluated several novel *Borrelia* and tick antigens as vaccine candidates, with initial promising results. Future *in vitro* and *in vivo* studies are planned for additional antigens in the pipeline. By eventually combining the most promising pathogen- and tick-derived antigens in a single cocktail vaccine, we aim to design a novel, broadly protective tool to combat LB and other TBDs simultaneously.
Introduction
In the Netherlands, Lyme borreliosis (LB) is not a notifiable disease, making surveillance difficult. A better understanding of the seroprevalence and geographical distribution of LB can lead to a more efficient strategy for disease control.

Objections/Aims
This study aimed to determine the seroprevalence of Lyme borreliosis in the Netherlands and identify associated risk factors for seropositivity.

Methods
Participants (n= 5,592, aged 0 to 89 years), enrolled from a population-based cross-sectional serosurveillance study conducted in 2016-17, provided a blood sample and completed a questionnaire. The presence of Borrelia-specific antibodies was determined, using a two-tier test strategy: screening was performed with an enzyme-linked immunosorbent assay and reactive sera were confirmed using an immunoblot. Seroprevalence was estimated for the Dutch population, taking into account the survey design. Furthermore, risk factor analyses were performed by using multivariable logistic regression models, in order to identify behavioral or intrinsic factors associated with seropositivity.

Results
In 2016-17, the seroprevalence for LB in the Netherlands was 4.4% (95% CI 3.5 - 5.2). Seroprevalence was higher in males (5.7%; 95% CI 4.4 - 7.2) compared to females (3.1%; 95% CI 2.3 - 4.0), increased with age (1.5% to 13.8%) and ranged regionally from 3.7% to 5.2%. The explanatory variables were tested in an univariate model and identified 32 variables as possible risk determinants for seroprevalence, among which gender, age, geographic region, number of tick bites in the last 5 years and level of urbanization. The multivariable logistic regression model fitted with the inclusion of the parameters that showed a potential association (p<0.1) in the univariate model is currently under evaluation.

Conclusions
The seroprevalence for LB in the Netherlands is 4.4 %, being in line with reported seroprevalence rates in Western European countries. Higher seroprevalence in males and older people may be associated with higher exposure rate as was also shown by tick bite rate.
PP08 - The Cdkn2a gene product p19 alternative reading frame (p19ARF) is a critical regulator of IFN-mediated Lyme arthritis

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Ecology, September 6, 2022, 10:50 AM - 12:50 PM

Introduction
Lyme disease, caused by infection with the tick-borne spirochete Borrelia burgdorferi, is responsible for a spectrum of clinical disorders in patients. The contrast in disease severity can be studied using C3H and C57BL/6 mice. Severe arthritis in C3H mice requires the exaggerated production of Type I IFN, and is reduced following antibody or genetic disruption of the type I IFN receptor. Elevation in type I IFN has also been observed in Lyme disease patients.

Aims
To positionally clone the polymorphic gene responsible for elevated Type I IFN production associated with severe Lyme arthritis in C3H mice.

Methods
Forward genetics was used to identify quantitative trait loci associated with severe Lyme arthritis in C3H and B6 mice. The C3H allele of Bbaa1, which encompasses the type I IFN gene cluster, was strongly penetrant when introgressed onto B6 mice; B6.C3-Bbaa1. Advanced congenic lines allowed physical reduction of Bbaa1 to 2 Mbp and revealed 6 candidate genes. RNA silencing was employed to identify the regulator of Type I IFN expression in macrophages, which was confirmed with B. burgdorferi infected mice.

Results
RNA silencing identified the Cdkn2a gene as the sole regulator of Type I IFN within Bbaa1, with an expression polymorphism in its p19ARF product found to be responsible for heightened expression of the C3H allele. Reconstitution of B6-Arf−/− mice with myeloid cells expressing the C3H-p19ARF allele developed severe arthritis, whereas those reconstituted with cells expressing the B6-p19ARF allele developed mild disease. p19ARF was found to enhance IFNb induction by several bacterial PAMPs, however, not by the viral mimic poly I:C, suggesting it could be targeted in Lyme arthritis without compromising anti-viral responses. p19ARF regulation of IFNb expression was mediated by the tumor suppressor p53 and the transcription repressor BCL6, thus providing additional potential targets for intervention in Lyme arthritis. The involvement of BLC6 was confirmed in B. burgdorferi-infected mice.

Conclusion
We have identified the novel involvement of p19ARF, p53, and BCL6 in modulating IFNb expression in Lyme arthritis development. These proteins provide potential therapeutic targets for the treatment of Lyme arthritis without generalized immune suppression.
Introduction
In regions endemic for *Borrelia burgdorferi*, Lyme disease is a common cause of knee monoarthritis. However, distinguishing Lyme arthritis from other conditions such as septic arthritis and other clinical mimics in children remains difficult.

Methods
We prospectively enrolled children with knee monoarthritis presenting to one of eight emergency departments participating in Pedi Lyme Net clinical research network (2015-2019). Using research biosamples collected in Tempus™ tubes, we performed whole blood RNAseq analysis. We compared the whole-blood transcriptional signature of children with Lyme monoarthritis of the knee (n=65) to age, sex, race and ethnicity matched healthy controls (n=65) as well as children with sterile knee monoarthritis of another cause (n=65) and septic arthritis (n=15). We divided the sample into a derivation (two-thirds) and validation (one-third) cohort for the evaluation of the transcriptional signature.

Results
More than 1500 transcripts distinguished Lyme arthritis from healthy controls at a false discovery rate (FDR) of 0.05. However, almost all of these transcriptional changes were shared with arthritic controls, with only a single transcript (IL21-AS1) that encodes IL-21 antisense RNA 1 that differentiated children with Lyme disease from other monoarthritis at an FDR of 0.05. This finding remained robust in an independent validation cohort of 25 patients per group, with an AUC of 0.81 [95% confidence interval (CI) 0.72-0.91] in the derivation cohort and 0.77 (95% CI 0.63-0.90) in the validation cohort to distinguish Lyme knee monoarthritis from sterile knee monoarthritis of another cause. The IL21-AS1 signature was observed across genders and age groups. However, IL21-AS1 failed to distinguish Lyme monoarthritis from septic monoarthritis, restricting the diagnostic utility of this signature to children in which bacterial joint infection is considered unlikely.

Conclusion
Together these results define the peripheral blood transcriptional signature of Lyme arthritis in children and illustrate the essential role of clinical mimics in the evaluation of diagnostic transcriptomics.
Introduction

*Borrelia burgdorferi* sensu stricto (Bb) is a tick-borne spirochete that causes Lyme borreliosis. In nature, populations of Bb consist of genetically distinct strains that co-exist in the same vertebrate reservoir host and tick populations. These Bb strains differ in their frequency, but the reasons why some strains are more common than others are not well understood.

Objectives/aims

The purpose of this study was to investigate whether strains that establish high abundance in host tissues have higher transmission success to feeding ticks.

Methods

For each of 11 strains of Bb, we experimentally infected 8 C3H/HeJ mice via nymphal tick bite. Mice were infested with *Ixodes scapularis* larvae on days 30, 60, and 90 post-infection (PI) to determine the lifetime host-to-tick transmission success of each strain. The engorged larvae were allowed to moult into nymphs and the nymphs were frozen at 4 weeks post-moult. The mice were euthanized at 97 days PI and 7 organs were dissected. All organs and xenodiagnostic nymphs were tested for the presence and abundance of Bb using qPCR.

Results

We found significant differences among the 11 strains in the abundance of Bb in the mouse organs; this phenotype differed 4.8-fold between the highest and the lowest strains. We also found significant differences in host-to-tick transmission success among the 11 strains, the best strain infected 1.8 times more ticks than the weakest strain (98.7% versus 54.3%). Most importantly, we found that strains with the highest abundance of Bb in the mouse organs also had the highest transmission success to feeding ticks.

Conclusions

Our study suggests that strains of Bb are under strong selection to maintain high abundance in those mouse tissues that facilitate transmission to feeding *Ixodes* ticks.
Determining effects of winter weather conditions on nympha \textit{Ixodes scapularis} and adult \textit{Amblyomma americanum} survival in Connecticut and Maine, USA

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Ecology, September 6, 2022, 10:50 AM - 12:50 PM

\textbf{Introduction}

Due to factors such as habitat modifications, altered host dynamics, and climate change, existing/emerging ticks and tick-borne diseases are becoming increasingly more prevalent. \textit{Ixodes scapularis} have not only expanded their range within the northeastern USA, but also have increased in density. Of equal concern are endemic species that are expanding their range northward, establishing in areas that were previously inhospitable. It has been speculated that milder winter conditions play a direct role in increased survival and abundance of native, exotic, and emerging species. This study attempted to evaluate the effects overwintering conditions have on ticks of the northeastern USA. We hypothesized that impacts from climate change have allowed endemic species to survive winters in greater abundances while also permitting the ability to expand their range and distribution.

\textbf{Methods}

The study occurred over three winters in Connecticut (CT) and Maine (ME), USA. Insulative barriers (leaf litter and snow accumulation) were manipulated to determine which were more vital to overwintering survival. To contain and monitor the two tick species, 24 plastic 4-L pots were placed in the ground and backfilled with excavated soil. Holes were cut in the top and bottom of pots and screened to make interior conditions comparable to exterior. Each pot contained plastic, screened, cylindrical vials with 5 laboratory-reared \textit{Amblyomma americanum} males (n=3), 5 females (n=3), and 15 nymphal \textit{I. scapularis} (n=3). Pots were randomly assigned one of four treatments: leaf removal (LR), snow removal (SR), both (LRSR), or control.

\textbf{Results}

Nymphal \textit{I. scapularis} had significantly decreased survival in the LR and LRSR treatments as compared to control (p=0.045 and p=0.008, respectively). Key overwintering predictors for nymphal \textit{I. scapularis} survival were within year mean and mean minimum temperatures. Both sexes of \textit{A. americanum} had increased survival in CT over ME (female p=0.004; male p<0.001), increased survival between years in ME (female p=0.017; male p=0.014), and females experienced increased survival in CT (p=0.033). For \textit{A. americanum} (as compared to \textit{I. scapularis}), presence or absence of snow and/or leaf litter had no impact on survival. Overall, we found a positive correlation between mean hourly temperature and adult survival in ME where winter soil temperatures were consistently below freezing as compared to CT where sub-freezing soil temperatures were anomalous.

\textbf{Conclusion}

We determined that ground-level conditions and minimum temperatures play an important role in nymphal \textit{I. scapularis} and adult \textit{A. americanum} overwintering survival. The results of this study can be included in predictive analytic models that can complement adaptive management strategies to accommodate expected fluctuations and range expansion of species such as \textit{I. scapularis} and \textit{A. americanum} that will most likely accompany an increase in temperatures throughout the Northeast over time.
PP12 - Secretoglobin family 1D member 2 (SCGB1D2) protein inhibits growth of Borrelia burgdorferi and affects susceptibility to Lyme disease

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Ecology, September 6, 2022, 10:50 AM - 12:50 PM

Introduction
The host factors that modulate susceptibility for Lyme disease have remained mostly unknown. In the context of Lyme disease, investigating the host-pathogen interactions in the skin, at the site of infection very important. Here we show a novel host defense mechanism against Lyme disease in humans through a variant identified through patient genetics which has allowed us to identify a protein that is secreted by the sweat glands in human skin and inhibits growth of Borrelia burgdorferi (Bb) in culture.

Methods
We utilized data from 342,499 individuals in who have participated in the FinnGen project to estimate the effect of genetic variation for Lyme disease. 5,248 (1.5%) of FinnGen participants had received a Lyme disease diagnosis between 1988 and 2021 as identified through ICD-codes in the Finnish national hospital and primary care registries. We then tested the function of the proteins identified in this analysis when combined with exponential phase cultures of Bb.

Results
Using epidemiological and genetic data from FinnGen, we identify a common missense variant at the gene encoding for Secretoglobin family 1D member 2 (SCGB1D2) protein that increases the susceptibility for Lyme disease. The genetic variant changes proline at position 53 to leucine and is predicted as deleterious. Consequently, we validate the dysfunction of this protein variant using live Bb. Recombinant reference SCGB1D2 protein inhibits the growth of Bb twice as effectively as the recombinant SCGB1D2 P53L deleterious missense variant.

Conclusion
Together, these data suggest that SCGB1D2 is a host defense factor present in the skin, sweat, and other secretions which protects against Bb infection. This discovery suggests that there could be a therapeutic or preventative pharmaceutical application for this secretoglobin protein which we will continue to investigate in mice and in culture.