Inferring tick movements at the landscape scale by SNP genotyping

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13TH INTERNATIONAL CONFERENCE ON LYME BORRELIOSIS AND OTHER TICK BORNE DISEASES

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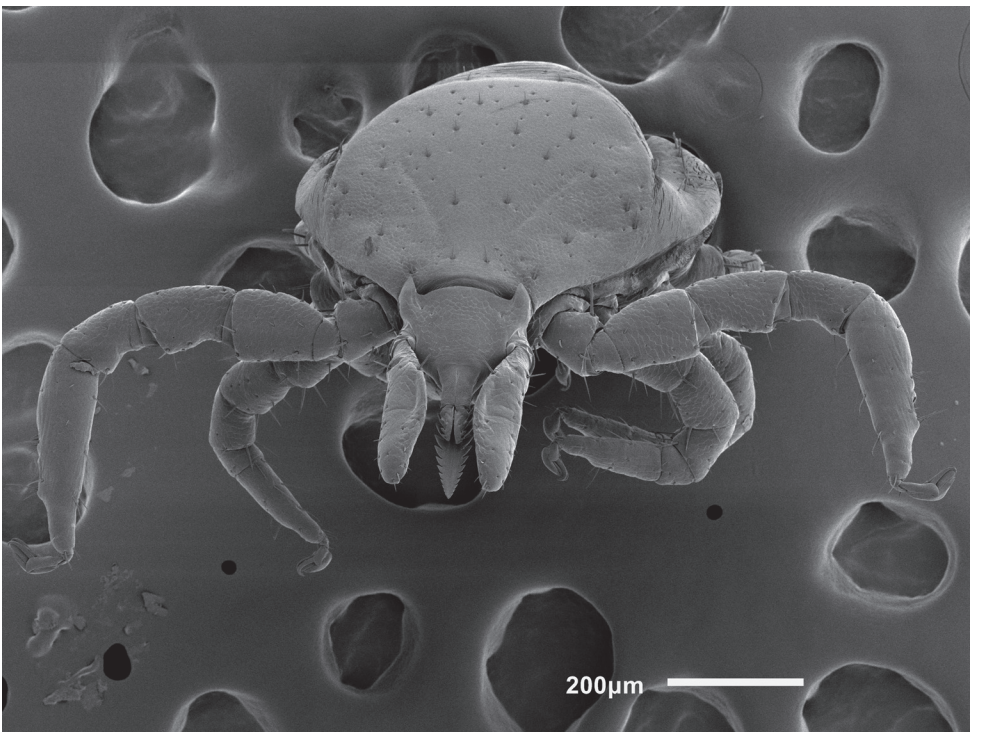

13th International Conference on Lyme Borreliosis and other Tick Borne Diseases Abstracts

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Summary of the Thirteenth International Conference Lyme Borreliosis and Other Tick-Borne Diseases

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Within this issue of Frontiers are abstracts from the Thirteenth International Conference on Lyme and Other Tick-Borne Diseases (ICLB), held August 18–21, 2013 in Boston, Massachusetts. ICLB is one of the preeminent conferences in the world for clinical, epidemiological, and pathogenetic studies of tick-borne diseases. The first conference was held in November, 1983, at Yale University in New Haven, CT, and focused on Lyme borreliosis, which had recently been identified as an *Ixodes* tick-transmitted spirochetal infection. Since then, the conference has been held every 2–3 years alternating between sites in the United States and in Europe. Although Lyme borreliosis remains the most common vector borne-disease in the United States and in Europe, the subject matter of the conference has expanded to include other important agents of human disease that are transmitted by *Ixodes* sp. ticks including but not limited to *Anaplasma, Ehrlichia*, *Babesia* species, relapsing fever spirochetes, and tick borne encephalitis virus. The Thirteenth ICLB conference brought together some of the most knowledgeable investigators in the world along with their trainees to share scientific discoveries relevant to infections transmitted by *Ixodes* ticks. Attendees came from a diversity of backgrounds including physicians, clinical and laboratory-based researchers, veterinarians, ecologists, and entomologists from academia, industry, and government, representing 26 countries in North America, Europe, and Asia. There were over 250 participants presenting 220 posters.

The conference opened with a celebration honoring the careers of two physician scientists – Stephen Malawista[1] and Allen Steere – whose investigation of childhood arthritis ultimately led to the discovery of Lyme disease, the first *Ixodes* tick-borne disease identified in the United States. Malawista, whose scientific career was dedicated to the study of inflammation and blood leukocytes in human disease, especially in gout and Lyme disease, was Chief of the Section of Rheumatology at Yale. Steere, a CDC-trained epidemiologist, had just begun his rheumatology postdoctoral fellowship training and was poised to "get to the bottom" (his own words) of what was causing Lyme arthritis, a research endeavor to which he has devoted his entire career. To honor them both, a poster entitled "A Newspaper Survey of The Discovery of Lyme Disease" was displayed to chronicle the remarkable epidemiologic efforts that culminated in the identification of an emerging *Ixodes* tick-borne spirochetal infection the United States.

As can be seen in the abstracts that were presented orally and in poster format, the conference covered a range of topics, from ecology and epidemiology, to new information about the basic biology of Lyme *Borrelia* and other tick-borne pathogens, to disease pathogenesis and host immunity, to clinical presentations, diagnostics, interventions, and outcomes. Three themes in particular were highlighted during the plenary sessions: (1) ways to curtail the spread of tick-borne diseases; (2) approaches to the question of Lyme *Borrelia* persistence in humans after treatment; and (3) priorities for future research of tick-borne diseases.

The opening plenary lecture was delivered by the entomologist Durland Fish, who described environmental strategies to limit the spread of tick-borne diseases. Although such measures can be effective, he cautioned the audience on the need to act quickly, as their implementation is easier before enzootic cycles have been firmly established. On-going active surveillance is essential for monitoring the potential threat of tick-borne diseases. New strategies for disrupting existing zoonoses are critical, as we learned from Paul Mead of the Centers of Disease Control and Prevention that the estimated annual incidence of Lyme disease in the United States is about 300,000 cases. In addition to Lyme disease, *Ixodes* ticks transmit other pathogens of human concern, including *Anaplasma phagocytophilum*, *Babesia* species, tick-borne encephalitis virus, and the newly emergent relapsing fever spirochete Borrelia miyamotoi. Regarding the latter, Platonov and our Russian colleagues reported the range of clinical and epidemiologic features 128 Borrelia miyamotoi cases – the largest documented series in the world.

A special plenary session was devoted to the issue of bacterial persistence. James Collins from Boston University gave a scintillating presentation on bacterial "persisters," defined as dormant cells within a genetically homogeneous bacterial population that are tolerant to the effects of antibiotics. Excessive use of antibiotics may actually drive bacteria to a state of persistence, contrary to a popular lay notion that long-term therapy is the solution to their eradication. A panel discussion ensued in which Mark Klempner reviewed the clinical trial data showing lack of efficacy of extended courses of antibiotics for people with post-treatment Lyme disease syndrome; Stephen Barthold discussed the results of animal studies suggesting Lyme *Borrelia* persistence; Linda Bockenstedt explained the importance of using tick-borne infection (ideally with a single nymphal tick) rather than cultured inocula to study Lyme *Borrelia* persistence in animal models if the goal is to gain insight into human disease; Monica Embers reported preliminary results of a study using tick-transmitted infection in the rhesus macaque Lyme borreliosis model; and

1 Malawista passed away on September 18, 2013, after a five-year battle with metastatic melanoma.

Brian Fallon raised questions about the etiology of neurocognitive complaints in people with post-treatment Lyme disease syndrome and how best to study them. Although no answers were provided, the group discussion provided a forum for researchers to clarify issues and identify concerns.

The conference concluded with summaries from each of the session chairs regarding future directions in the field. The general lack of funding for training the next generation of ecologists/entomologists in the field of tick-borne diseases was raised, particularly in light of the anticipated retirement of many long-standing leaders in this area. The need for better prevention strategies for tick-borne diseases was highlighted, with some calling for re-release of the LymeRx vaccine and others suggesting that drastic measures may be necessary if outbreaks of Powassan virus become more prevalent now that the virus has expanded to *Ixodes* sp. ticks that transmit Lyme disease. Finally, the need to conduct studies in humans to gain more information about human biology and how it impacts the outcome from tick-borne diseases was conveyed.

Overall, the conference was considered a great success. The feedback from attendees was uniformly positive, which Linden and I can only attribute to the quality of the science and the participation of the attendees themselves. We are grateful for the opportunity to continue this biannual tradition, started in 1983 by Steere, and look forward to the next conference in Vienna, Austria in 2015.

003

Inferring tick movements at the landscape scale by SNP genotyping

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Assessment of tick movements is particularly important for the understanding of the eco-epidemiology of tick-borne-diseases and for the design of control methods against this vector and transmitted pathogens. Tick dispersal is mainly due to host movements. However, because of the high diversity of *Ixodes ricinus* hosts, to which is associated a wide range of dispersal abilities (ranging from a few meters for rodents to hundred of kilometers for birds) and of the unknown relative contribution of the different host species in tick feeding, estimating tick dispersal is a particularly challenging task. Moreover, capture-mark-recapture methods cannot be used for those small animals with their growth interrupted by molting. Thus, population genetics – through the assessment of gene flow – provides a particularly valuable tool to estimate tick dispersal. Unfortunately, the microsatellite loci – considered as the most powerful markers for populations genetics studies due to their hypervariable and co-dominant features – developed until now for *I. ricinus* exhibit heterozygous deficiency and null alleles that are harmful for any population genetics investigations. To circumvent this caveat, we have developed a set of 384 single nucleotide polymorphisms (SNPs). The genotyping of those bi-allelic markers is unambiguous and straightforward and the large number of loci allows a wide coverage of the polymorphism in the whole *I. ricinus* genome. Because of the scarcity of genomic resources for *I. ricinus*, we have conducted a partial sequencing of its genome to isolate SNPs. Among the 1.2 millions of reads obtained (average length 540 bp), SNPs were isolated using an original bioinformatic method. A set of three primers have been designed for each individual SNP locus. Finally, a Fluidigm array was used to allow the genotyping of individual ticks on 384 different SNPs. Five hundred questing nymphs – individually georeferenced – collected in a landscape (including forests, hedges, and pastures) from western France were genotyped. The analysis of the variability of tick populations with landscape genetics tools will be discussed. Moreover, each genotyped tick is also analyzed to identify the host used for its previous blood meal, and *Anaplasma phagocytophilum*, *Borrelia bugdorferi*, as well as *Babesia divergens* are looked for and characterized molecularly. It is thus possible to investigate potential associations of tick genotypes with particular hosts, pathogens, or biotope. This is the first study reporting the development of primers allowing the genotyping of SNPs in *I. ricinus* and of landscape genetics of any ticks.

004

Tick adverse moisture events (TAMEs) determine nymphal blacklegged tick encounter risk

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Ixodes scapularis nymphs, the primary Lyme borreliosis vector stage in the eastern United States, displays sensitivity to conditions of low environmental moisture, with increasing tick mortality demonstrated under longer exposures to low relative humidity conditions in the laboratory. However, field studies examining the relationships between environmental moisture, tick encounter risk, and Lyme borreliosis incidence have generated conflicting results. We examined the relationship between tick adverse moisture events (TAMEs), defined for nymphal *I. scapularis* as >8 h of continuous <82% near-ground relative humidity (RH) and tick encounter risk using 14 years of tick surveillance data from Rhode Island (USA). The total number of TAMEs occurring in June of each year was negatively related to total seasonal nymphal *I. scapularis* encounter risk during that year, suggesting that extended lower humidity periods may serve to reduce nymphal blacklegged tick populations. Linear regression of the total number of TAMEs during June successfully predicted total seasonal nymphal tick encounter risk for the same year ($P \leq 0.027$). Furthermore, the number of TAMEs occurring during June was positively related to the ratio of tick counts collected in early season compared to late season ($P = 0.040$), suggesting that the significant TAME population effect was due to tick mortality. Multiple regression of a four-parameter model incorporating June humidity and degree of winter severity on the 2 year life-cycle of *I. scapularis* did not improve the analysis ($P = 0.660$). Results provide insight into the means by which environmental moisture influences tick survival. Moreover, findings offer the possibility of increasingly accurate predictability of tick encounter risk and improved targeting for tick-borne disease preventive measures.

005

Genetic variability of *Anaplasma phagocytophilum* and its implication in the ecology of anaplasmosis in Central Europe

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Anaplasma phagocytophilum is the causative agent of granulocytic anaplasmosis of both medical and veterinary importance. In Europe, *A. phagocytophilum* is transmitted by the Ixodid ticks, mostly *Ixodes ricinus*, however, other tick species such as *Ixodes trianguliceps* may contribute to the enzootic cycle of this bacterium. It is maintained in nature by several vertebrate hosts. Genetic analyses of *A. phagocytophilum* strains can reveal ecological forces that have shaped their genetic diversity, such as the extent of the pathogen vector- and host-specialization. In order to understand the ecology of this pathogen in Central Europe, we have analyzed and compared the genetic variability of different *A. phagocytophilum* strains from questing and feeding ticks collected from vegetation and different vertebrate hosts (roe deer, rodents, birds, sheep, and dogs), as well as blood and biological samples of some vertebrate hosts (rodents and birds), from several sites in two regions of Central Europe (Slovakia and Northern Italy). *A. phagocytophilum* was detected in questing and host feeding *I. ricinus* ticks from all studied sites, as well as from feeding *I. trianguliceps* on rodents and rodents' ear and spleen biopsies. Prevalence of *A. phagocytophilum* in areas with rodents was significantly much lower than in areas without rodents. In areas where *I. trianguliceps* ticks were absent, we did not detect *A. phagocytophilum* in rodents. Phylogenetic analysis based on four genetic loci of *A. phagocytophilum* positive samples have shown that *A. phagocytophilum* genotypes in questing *I. ricinus* and feeding *I. ricinus* from ungulates, birds, and dogs were distinct from genotypes found in rodents and feeding *I. trianguliceps*. The Msp4, DOV, and GroEL sequences of *A. phagocytophilum* genotypes showed considerable heterogeneity but none of the positive questing *I. ricinus* tick was found infected with the rodent genotype that was identical to the genotype detected in *I. trianguliceps*. The GroEL sequences from rodents were closely related to the homologous sequences found in previous studies in questing *I. persulcatus*. Our study from central Europe confirms the previous findings from UK that *A. phagocytophilum* strains have specific associations with two vectors and different reservoir hosts. Unlike in the US, *A. phagocytophilum* genotypes that are associated with rodents are probably transmitted solely by *I. trianguliceps* ticks, therefore these strains may be not of risk for humans, considering the narrow host selection behavior and feeding preference of this tick species.

006

Trends in news reports about ticks and the infections they transmit: analysis of The NY Times archives, 1870–2012

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Ticks and their associated pathogens/maladies have plagued man and beast for thousands of years. While there may be instances of occasional references pertaining to ticks in historical writings, first-hand descriptive accounts of how ticks have affected people, their livestock, and even their pets are rare, if not absent, from our collective conscience prior to the industrial age. The digital-age has transformed many areas of modern life and one of these is the opportunity to copy (scan), store, and quickly sort through large quantities of recorded information. In that vein, The New York Times has digitized a large portion of their US newspaper archives dating back to the 1860s rendering them "searchable" by key words for analysis and reflection. This author became fascinated by the possibility to mine these digital archives of newspaper references and articles pertaining to how ticks have impacted American society over the past 150 years. The intent of this brief oral abstract will be to trace the arc of human concern/observation relating to ticks in our society. By means of a year-by-year sweep of daily life in the newspaper since the 1860s, this paper will report on how ticks have contributed to human concern, anxiety, and financial loss, be it in the agricultural setting (cattle), in new disease discoveries (cattle and human), and their consequences on human/companion animal health.

007

Positional cloning identifies β-glucuronidase as a key regulator of murine Lyme arthritis and rheumatoid arthritis

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Lyme disease is the most prevalent arthropod-borne illness in the US. Up to 60% of patients develop inflammatory arthritis following infection. Host genetics play a key role, as inbred mouse strains exhibit consistent differences in Lyme arthritis severity. Our lab previously described maximal linkage of disease (LOD, 10.2) severity to the *Bbaa2* locus on mouse chromosome 5. Recently, we developed 15 advanced congenic mouse lines carrying sub-intervals of *Bbaa2* from susceptible C3H on an otherwise uniform resistant B6 genetic background. This led to the positional cloning of a novel disease regulator, the lysosomal enzyme β-glucuronidase (*Gusb*). C3H mice harbor a coding-non-synonymous point mutation in the Gusb gene and are Gusb hypomorphs, exhibiting a 90% enzymatic deficiency. Two sub-strains of CBA mice carrying the hypomorphic or wild type *Gusb* alleles develop severe Lyme arthritis or are protected, respectively. A *Gusb-null* mouse strain on the B6 background develops severe Lyme arthritis, while heterozygotes are protected. Likewise, heterozygous congenic mice carrying one copy of the C3H *Gusb* deficiency allele are also protected. Transgenic overexpression of *Gusb* in C3H mice to correct the defect led to a profound and highly significant reduction in Lyme arthritis severity, indicating that *Gusb* is a key regulator in this strain. *Gusb* congenic mice also develop more severe disease in the KBxN model of rheumatoid arthritis, suggesting a conserved role. Experiments with radiation chimeras indicate that the lysosomal pool of *Gusb*, not serum level, regulates disease severity and that residual joint resident cells primarily mediate the effect. Severe deficiencies in *Gusb* and many other lysosomal enzymes are known to manifest themselves clinically as lysosomal storage diseases with joint involvement, implying a shared mechanism of pathogenesis.

008

The role of integrins in vesicular trafficking and immune responses to *Borrelia burgdorferi*

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Host cell responses are tailored to the infecting microbe. Although a relatively simple concept, a continuing area of research has been the elucidation of how a handful of immune receptors generate such a wide range of inflammatory signatures. Cell biology has been shown to be crucial in customizing these responses. Depending on cellular location, signaling results in marked differences in cytokine profile. Our studies with *Borrelia burgdorferi* have allowed us to contribute to a shifting paradigm in our understanding of the role of cellular compartmentalization in Toll-like receptor (TLR2) signaling. In our current work, we explore the relevance of specific motifs within cell surface molecules and the role of intracellular trafficking molecules to define internalization and sorting into intracellular compartments. We show that the β1 integrin affects *B. burgdorferi* dependent TLR2 signaling and internalization of the pathogen through the NPXY motifs in its cytoplasmic domain. Mutations in the tyrosine residue in two of these motifs significantly decrease signaling of *B. burgdorferi* and TLR2 ligands. In addition, we show that this decrease is due to a defect in internalization of *B. burgdorferi* and TLR2-ligands attached to beads. Thus, the NPXY motif of the β1 integrin serves as an internalization signal for TLR2 ligands and *B. burgdorferi*. Upon internalization of *B. burgdorferi* or TLR2 ligand-beads, adaptor protein complexes in the endosomal vesicular pathway play an important role in inflammatory responses induced by these ligands, as cells from knockout mice show a significant decrease in either TLR2 ligand-beads or *B. burgdorferi*-induced signaling. The mode of internalization is crucial to proper trafficking since free TLR2 ligand is not affected in the same way as *B. burgdorferi* or TLR2 ligand-beads. Our study is the first to identify a role for the integrin NPXY motif in the internalization of *B. burgdorferi* and TLR2 ligands and describe vesicular trafficking of *B. burgdorferi* and TLR2.

009

Regulation of *Borrelia burgdorferi*-induced arthritis by TAM receptors

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Toll-like receptor (TLR) mediated recognition of *Borrelia burgdorferi* (Bb) components initiate Bb-induced inflammatory responses, which include production of type 1 IFN. In the murine model of Lyme borreliosis, arthritis susceptibility in C3H mice has been linked to robust type 1 IFN expression levels and neutralization of its effects ameliorates disease. Although much is known about the mechanisms through which Bb incites inflammation, relatively little is known about the endogenous pathways that regulate the severity of the inflammatory response to Bb, particularly in the disease-quiescent phase of infection. Recent studies have shown that the Tyro3/Axl/Mer (TAM) family of receptor tyrosine kinases plays a critical role in clearance of apoptotic cell debris and in reducing inflammation associated with TLR stimulation and type 1 IFN cytokine production. In this study, we used *mer*$^{-/-}$ and *axl*$^{-/-}$ *mer*$^{-/-}$ mice to assess effects of these deficiencies on Bb infection and arthritis. Bone marrow-derived dendritic cells (DCs) and macrophages (DMs) from B6 and C3H mice exhibit increased expression of *ax*l and *mer* mRNA within 24 h of *in vitro* stimulation with Bb lysate. TAMs exert their anti-inflammatory effects through induction of *socs*1/3 expression, and Bb-induced *socs* expression as assessed by qRT-PCR was impaired in DMs and DCs from *axl*$^{-/-}$ *mer*$^{-/-}$ mice. Although B6 *mer*$^{-/-}$ mice infected for 14, 21, or 46 days had equivalent arthritis incidence and severity as B6 WT mice, B6XC3H (F1) *mer*$^{-/-}$ mice exhibited significantly enhanced arthritis at 21 days of infection. No differences in pathogen burden assessed by qPCR for Bb *recA* or Bb-induced antibody titers were found. Effects of TAM deficiency on Bb infection were more apparent in *axl*$^{-/-}$ *mer*$^{-/-}$ mice, with arthritis present in 12/31 B6.129 axl$^{-/-}$ mer$^{-/-}$ joints and 0/27 B6.129 control joints by histopathology at 21 days of infection. Arthritis persisted in B6.129 *axl*$^{-/-}$ *mer*$^{-/-}$ mice at least until 42 days of infection, the latest time point examined. Two-photon intravital imaging of the patellar entheses at this late time point revealed enhanced deposition of amorphous GFP deposits in infected *axl*$^{-/-}$ *mer*$^{-/-}$ joints (4 of 5 mice examined) in comparison to infected WT joints (0 of 6 mice examined). Taken together, these findings indicate that the TAM pathway may serve to limit Bb-induced inflammation and to facilitate clearance of Bb antigenic remnants that may be deposited in the entheses during infection.

010

Dysregulation of CD4+CD25HI+ T cells in the synovial fluid of patients with antibiotic-refractory Lyme arthritis

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Background: Lyme arthritis, a late stage manifestation of Lyme borreliosis, results from infection with the tick-borne spirochete *Borrelia burgdorferi* (Bb). Lyme arthritis usually resolves after spirochetal killing with antibiotics (antibiotic-responsive arthritis). However, some patients have persistent synovitis that lasts for months or years despite >3 months of antibiotic therapy (antibiotic-refractory arthritis). This latter outcome is postulated to result from either persistent infection, retained spirochetal antigens, or infection-induced autoimmunity. Regardless of the antigenic driver(s) behind this illness, we postulate that this subgroup of patients is unable to properly down-regulate their immune response leading to immune dysregulation and persistent synovitis.

Methods: Immune dysregulation in refractory patients was assessed by comparing the phenotype, frequency, and function of CD4+ T effector (Teff) and T regulatory (Treg) cell populations in the synovial fluid (SF) of these two patient groups. Samples from antibiotic-responsive patients ($N = 15$) were obtained during infection, whereas those with antibiotic refractory patients ($N = 16$) were obtained during the post-antibiotic period when immune dysregulation is thought to play a critical role. Immune cells were analyzed using flow cytometry and suppression and cytokine assays.

Results: Significant differences between the two patient groups were found in the CD4+CD25hi+ population, a population of cells usually composed primarily of Treg cells. Refractory patients had substantially fewer Foxp3-positive Treg cells and an enrichment of Foxp3-negative Teff cells in the CD25hi+ cell population was compared with responsive patients. Additionally, CD4+CD25hi+ cells in refractory patients had significantly greater expression of GITR and OX-40, two co-receptors that augment T cell function. Suppression assays showed that CD4+CD25hi+ T cells in patients with refractory arthritis did not effectively suppress proliferation of CD4 + CD25-negative T cells or the secretion of the inflammatory cytokines IFN- and TNF, whereas those from patients with responsive arthritis did. Finally, in refractory patients, higher ratios of CD25hi+Foxp3-negative/CD25hi+Foxp3-positive cells correlated directly with longer durations of arthritis.

Conclusion: Patients with antibiotic-refractory had lower frequencies of Treg cells in SF and greater expression of T cell co-receptors that are known to enhance Teff resistance to suppression compared to patients with antibiotic-responsive arthritis. These lower frequencies of Treg were unable to effectively suppress Teff cells leading to immune dysregulation and persistent synovitis. Additionally, in refractory patients, higher Teff to-Treg cell ratios in SF were associated with longer post-antibiotic durations of arthritis. These data suggest that the immune responses in these patients were highly augmented leading to immune dysregulation and an antibiotic-refractory Lyme arthritis outcome.

012

Delineation of *Borrelia burgdorferi* dissemination kinetics and persistence within murine skin via intravital microscopy

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After transmission via tick-bite, *Borrelia burgdorferi* (Bb) must rapidly detect its new host and adapt such that it can disseminate and persist in the face of potent innate and adaptive immune responses. There is a great interest in identifying these critical adaptations that allow immune evasion, but as an obligate parasite, *in vitro* analyses have provided conflicting results. Thus, there is a great need to develop a more accurate system for assessing spirochete–host interactions *in vivo*. To address this, we have developed a confocal microscopy-based system that allows us to visualize fluorescent, virulent spirochetes within intact murine skin and in real-time. The goal of this study is to use these techniques to accurately describe Bb motility behaviors during early and late infection, the environments preferentially inhabited by the bacterium, and to observe interactions with skin-resident immune cells. Cutaneous inoculation of fluorescent Bb into murine ears revealed that a population of bacteria appeared to fragment and die within minutes of inoculation while other bacteria endured. Spirochete numbers were constant until 48–72 h post-inoculation, when rapid proliferation was observed and the bacteria began to disseminate from the injection site. Maximum Bb numbers were observed at day 5 post-infection followed by a 96-fold drop in numbers by day 12, which correlates with the induction of Bb-specific antibodies. Viable spirochetes disseminate through the dermis and appear to preferentially inhabit collagen-associated strata. Two major motility patterns were observed: backward–forward and extended run. Velocity measurements indicated that during early dissemination, Bb could achieve speeds of 1262 μm/min, while during late dissemination the bacteria exhibited only backward–forward motility with velocities of 237 μm/min. Parallel experiments using mouse strains possessing fluorescent immune cell subpopulations indicated that Bb did interact with Langerhans cells, but no phagocytosis events were observed. Other antigen-presenting cells were observed to contact and internalize Bb, and neutrophils migrated into infection foci within 3–6 h and also internalized substantial numbers of bacteria. However, the majority of spirochetes were able to escape, as their speeds were 40–100 times greater than the fastest immune cells. These findings suggest that Bb motility/chemotaxis is critical for pathogenesis and immune evasion at both early and late stages of infection, and that Bb displays constant motility through all phases of infection. Expansion of this imaging model is anticipated to allow true delineation of host–Bb interactions vital for Lyme disease progression and may subsequently reveal key targets for preventative and curative therapies.

013

Blood donor screening for *Babesia microti*: implications for blood safety

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Background: *Babesia microti* is a transfusion-transmissible intraerythrocytic parasite; incidence is increasing in the US and no FDA-licensed screening test is available. We conducted blood donor screening for *B. microti* using investigational antibody and DNA tests in selected counties of Massachusetts and Connecticut.

Methods: Arrayed fluorometric immunoassay (AFIA) and real-time PCR (IMUGEN, Norwood, MA, USA) were performed on EDTA-anticoagulated whole blood samples obtained from individuals presenting to donate at selected blood drives in MA and CT between June and November of 2012. The AFIA utilized native *B. microti* as an antigen substrate with reactivity defined as fluorescence at >1:128 dilution. Repeat AFIA-reactive (pos) samples were tittered to endpoint and tested by research Western blots. DNA was extracted using an automated magnetic bead isolation/purification system. PCR primers/probes targeted the *B. microti* 18S ribosomal RNA gene; the 50% detection limit is 10 piroplasms/mL. PCR-reactive samples were retested 3 times and those having ≥1 reactive result were considered positive. Initial PCR-reactive samples retesting reactive <3 times were termed inconclusive (inc). PCR-negative samples were tested using an enhanced sensitivity probit function PCR (ePCR). Quantitative PCR was used to determine parasite load; hamster pairs were inoculated to determine infectivity. Reactive donors were invited into a follow-up study for repeat testing and completion of a risk factor questionnaire.

Results: Of 19,625 donors screened, 100 (0.51%) tested pos/inc for *B. microti* antibodies and/or DNA, including five AFIA-neg/PCR-pos, 13 AFIA-pos/PCR-pos, and 80 AFIA-pos/PCR-neg. Two were inconclusive. Four PCR-neg units tested ePCR positive. Parasite loads were 13–870,000 piroplasms/mL; 11/16 hamster pairs developed signs of B. microti infection. Follow-up samples (117 from 82 donors) were obtained, confirming past/current infection; tick exposure (38/71, 53%); and time spent outdoors (68/71, 96%) were reported frequently. Recipient tracing of transfused red cells from prior donations from infected donors revealed 2/8 AFIA-pos recipients, but both reported other risk factors.

Conclusion: Blood donor screening in endemic areas is not only feasible, but also results in the removal of likely-infectious products from the blood supply.

014

Borrelia burgdorferi antigen epitope mapping and development of a multi-peptide seroassay for Lyme disease

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The two-tier system for the serodiagnosis of Lyme disease was established in the mid-1990s to address non-specificity inherent in whole or lysed *Borrelia burgdorferi*-based immunoassays. This was meant to be temporary until more specific technologies were developed; yet, despite a great deal of research, this system is still in use. Whole *B. burgdorferi* protein antigens, natural and recombinant, contain both epitopes unique to *B. burgdorferi* and epitopes that are similar to those from other bacteria. Peptide-based assays, such as the C6 assay, circumvent issues of nonspecificity by elimination of nonspecific epitopes. While the efficacy of a single-peptide based serological assay is limited by both antigenic variation and the variability inherent in the human immune response, a multi-peptide assay containing specific epitopes from several different *B. burgdorfe*ri antigens would avoid this limitation while maintaining superior specificity and sensitivity compared to current assays. We performed epitope mapping of the *B. burgdorferi* antigens OspC type K, OspC type A, FliLB, DbpA, DbpB, BmpA, OppA2, BBG33, LA-7, RecA, p66, Bbk32, OspF, p35, CRASP2, and ErpP. Overlapping peptide libraries consisting of 15 AA peptides overlapping by 10 AA were probed with sera from eight patients with clinically active Lyme disease, each of which had multiple well-defined bands on a commercial Lyme immunoblot strip. Multiple epitopes were detected for each antigen. Thirty-six peptides containing epitopes that were detected by a minimum of six of eight patient sera were further evaluated for reactivity with multiple serum samples derived Lyme disease patients presenting to Westchester Medical Center, Gundersen Lutheran Medical Center, or StonyBrook Medical Center. Sera from patients with rheumatoid arthritis and syphilis or from healthy individuals from Lyme disease endemic (southern New York) or non-endemic (New Mexico) areas were used as controls. Peptides from OspC type K (1), OppA2 (2), FlilB (2), Bbk32 (1), OspF (2),p35 (1), ErpP (1), DbpA (2), RecA (1), BmpA (1), and a previously identified modified IR6/FlaB chimera performed well in the initial analysis and are being further evaluated in combination. One such mixture, consisting of peptides derived from OspC, BmpA, OppA, FlilB, VlsE, and FlaB detected seropositivity in 50% of early LD sera obtained from patients upon first presentation with erythema migrans, while C6, the most specific and sensitive antigen, currently detected only 42%. We continue to evaluate peptide combinations with the goal of developing a seroassay containing five or more antigen targets.

015

Rapid point-of-care test for Lyme disease

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Introduction: The primary diagnostic approach for Lyme disease is based on serology, however, no serologic test is currently available in a rapid, point-of-care format. The ability to obtain accurate serologic results in a primary clinical setting would improve turnaround time for diagnosis, potentially enable resolution of uncertain cases, and reduce unnecessary antibiotic prescription where the diagnosis can be rapidly excluded.

Methods: A rapid test in a lateral flow format using a novel highly sensitive colorimetric detection technology was developed, using the C6 peptide augmented by an OspC-derived C10 peptide as antigens. The rapid test detects all antibody classes, takes less than 10 min to carry out from start to finish, and yields brightly colored test and control bands that can be read by eye. The test was evaluated on a panel of 207 serum specimens from patients with Lyme disease confirmed by positive skin or blood culture, of which 173 were from patients presenting with erythema migrans, and the remainder from patients with symptoms of disseminated infection including neuroborreliosis and Lyme arthritis. Rapid test results were compared with results from standard two-tier testing based on C6 ELISA and Lyme Western blots.

Results: Serum samples drawn from 59 patients within 1 week after appearance of single erythema migrans lesions were detected with 85% sensitivity by the rapid test vs. 49% sensitivity by C6 ELISA. For 29 multiple erythema migrans patient sera, sensitivities were 100% for the rapid test vs. 85% for the C6 ELISA. Sera from 22 patients with neuroborreliosis were detected with 100% sensitivity by the rapid test vs. 86% by C6 ELISA. Overall sensitivity among 173 patients with erythema migrans was 89% for the rapid test vs. 73% for the C6 ELISA. Analytical sensitivity of the rapid test was equal or better than that of separate ELISAs using C6 and C10 peptides. Specificity of the rapid test in 180 healthy blood donor sera was 100%.

Conclusions: The C6/C10 Lyme rapid test has demonstrated high sensitivity in detection of Lyme patient sera, significantly exceeding that of a comparable ELISA. The test may be especially useful in detection of very early stage Lyme disease where other assays are challenged in sensitivity. As a point of care test, the C6/C10 rapid test could offer clinicians an effective tool to enable more accurate in-office diagnosis of Lyme disease and may prove a suitable alternative to the standard two-tier testing algorithm.

016

Laboratory diagnosis of Lyme neuroborreliosis – a comparison of three commercial CSF anti-*Borrelia* antibody assays

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Background: Lyme neuroborreliosis (LNB) is the most common manifestation of disseminated borreliosis in Europe. The diagnosis is usually based on the medical history, clinical findings, pleocytosis in cerebrospinal fluid (CSF) together with anti-*Borrelia* antibody analyses in paired CSF, and serum samples. The aim of this study was to compare the widely used IDEIA Lyme neuroborreliosis test (Oxoid) using purified native flagellum as single test antigen, with two newly developed tests using several recombinant antigens for the laboratory diagnosis of LNB.

Materials and Methods: Serum and CSF from 199 patients investigated for suspected LNB during 2003–2007 were analyzed with the IDEIA Lyme neuroborreliosis (Oxoid), VIDAS Lyme IgG (bioMérieux), and recomBead Borrelia IgM/IgG (Mikrogen) assays. Intrathecal antibody indices (AI) were calculated as recommended by the manufacturers. Fifty-six of the patients had confirmed LNB, i.e., CSF pleocytosis and anti-*Borrelia* antibodies detected in CSF by Lyme Borreliosis ELISA Kit 2nd Generation (DAKO), the routine method used for laboratory diagnosis of Lyme borreliosis 2003–2007 at the Department of Clinical Microbiology, Jönköping, Sweden. Furthermore, the LNB patients were clinically evaluated regarding symptoms and response to antibiotic therapy, and they have been well characterized in previous studies. Forty-two patients had CSF pleocytosis but no anti-*Borrelia* antibodies detected by the DAKO test. The remaining 101 patients had no CSF pleocytosis and no anti-*Borrelia* antibodies detected with the DAKO assay in the CSF, and served as controls.

Preliminary Results: The IDEIA Lyme neuroborreliosis test performed with an overall sensitivity (both IgM and IgG AI) of 89% and a specificity of 100%. The VIDAS Lyme IgG test that measures anti-*Borrelia*-IgG AI only showed a sensitivity of 80% and a specificity of 99%. An overall sensitivity (IgM and IgG AI taken together) of 98% and a specificity of 100% were achieved by the recomBead Borrelia test.

Conclusions: We conclude that all three assays evaluated in this study performed very well regarding the specificity. However, the preliminary data suggest an improved diagnostic sensitivity with the recomBead Borrelia test as compared to the other two assays.

017

Proteomic analysis of cerebrospinal fluid distinguishes post-treatment Lyme syndrome as a neurologic entity

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The etiology of persisting symptoms, particularly fatigue and cognitive abnormalities, after *Borrelia burgdorferi* infection (CDC-criteria) remains unknown. Its existence as a biologic entity, independent of persisting infection, is controversial. To determine if there is a biological evidence for such symptoms, termed neurologic post-treatment Lyme syndrome (nPTLS), we performed proteomic analysis of cerebrospinal fluid (CSF) because it is akin to a "liquid window" of the brain, reflecting its microenvironment and the symptoms are referable to the central nervous system (CNS). We wanted to determine if the CSF proteome of nPTLS had (1) abnormal proteins and (2) proteins distinguishing nPTLS from normal and other diseases with fatigue and cognitive abnormalities. Our published normal CSF-proteome reference database (2586 proteins; 56% only in CSF, not plasma) allowed this. We used high-abundant protein separation, fractionation, and quantitative ultra-sensitive mass spectrometry (2D-LC–MS/MS) to characterize CSF from (a) nPTLS ($n = 30$) meeting CDC and NIH-trial criteria for Lyme initially, antibody to *Borrelia*, but no PCR or culture evidence of a persisting post-treatment infection, (b) normal ($n = 200$), (c) CDC-defined chronic fatigue syndrome (CFS) ($n = 45$), and (d) other neurologic diseases controls ($n = 20$). These methods have not been applied to Lyme CSF before. Quantitative differences in protein abundance revealed group-specific differences (p-value < 0.05) (Will show in figures/tables, with full protein names). CSF proteomes of each group contained 2500–2800 identifiable proteins; ~25% were group-specific. nPTLS had 2768 non-redundant proteins – 692 were nPTLS-specific. Both groups, and individuals within the groups, could be distinguished from the other groups by their specific CSF proteins ($p < 0.01$). Pathway analysis (Ingenuity) demonstrated data could be used for pathogenetic hypothesis generation. A few examples: Complement proteins (C1S, C4B, C1QB, C1QC) were significantly increased ($p = 0.005$) in nPTLS CSF compared to the others. CDK5-signaling pathway was significantly enriched ($p = 0.00009$) for proteins in CFS not nPTLS. Some proteins were decreased in both compared to healthy normals. However, quantitative distinguishing differences were found. In neural networks, e.g., axonal guidance, proteins in nPTLS were increased relative to CFS (ADAM23, EPHA7, PFN1, ROCK1). Our data demonstrate that nPTLS has abnormal presence of CSF proteins establishing that it is a neurologic entity and distinguishing it from other conditions with similar features even in the absence of microbiologic evidence of a persisting infection (suggesting it is related to a host response). CSF proteins found during this discovery phase may prove useful to develop and validate host biomarkers for this condition.

018

Metabolic biomarkers and biosignatures for improved diagnosis of Lyme disease

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Antibody-based diagnostics (EIA and WB) for Lyme disease have been widely used in laboratory confirmation for over 20 years. However, substantial limitations that include lack of specificity, sensitivity that is dependent upon the stage of infection, and the inability to distinguish between current and past infections persist. Additionally, results are often interpreted subjectively due to a lack of assay standardization. As an alternative to assessment of antibody profiles, we have applied metabolomics platforms for the discovery of biomarkers and biosignatures that could be used as diagnostics of infection, disease staging, prognostics, and indicators of cure. Specifically, we have analyzed by liquid chromatography–mass spectrometry (LC–MS) the metabolic profiles of human sera collected from patients diagnosed with Lyme disease and that fit into stage-specific categories of early localized Lyme, early disseminated Lyme, late Lyme, or cured Lyme. As control groups, sera from healthy individuals living in regions endemic and non-endemic for Lyme disease were also analyzed. Furthermore, sera from individuals with rheumatoid arthritis were included as controls against late Lyme disease. The LC–MS analysis of human sera resulted in the resolution of several thousand small molecule metabolites. The metabolic profiles of individual groups were compared using the Agilent Mass Profiler Professional software package. A defined set of metabolites with statistically different ($p < 0.05$) abundances between the following groups were repeatedly observed: early Lyme disease vs. healthy endemic controls, early Lyme disease vs. late Lyme disease, late Lyme disease vs. healthy controls, and late Lyme disease vs. rheumatoid arthritis. Additionally, overlapping metabolites that differentiate early Lyme disease from healthy endemic controls were identified within two separate analyses of two sample sets representing early Lyme disease. The identities of the small molecule metabolites that comprise the various biosignatures are being determined. To further assess the specificity of the biosignatures, sera from look-alike disease and syndromes (syphilis, infectious mononucleosis, multiple sclerosis, severe periodontitis, and fibromyalgia) are being analyzed.

019

Toward diminishing the Lyme *Borrelia* threat from nature: a prospective five-year field study report

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High prevalence of ticks infected with *Borrelia burgdorferi* has been linked to increased incidence of Lyme disease and areas of high prevalence-high risk have been mapped recently. Preventing the vector from acquiring or transmitting a pathogen is a useful strategy for preventing Lyme disease in humans. Subcutaneous injection of wildlife reservoirs with soluble OspA was previously shown to reduce vector infection prevalence with *B. burgdorferi* the year following intervention. Over the past 10 years, we developed and tested an OspA-based oral reservoir targeted vaccine (RTV) against *B. burgdorferi* and embarked on a comprehensive multi-year analysis to evaluate the effect of this baited vaccine on Lyme borreliosis over time. In Dutchess County, New York, seven plots were included in the field trial: four plots (NY1-NY4) were treated with RTV; three control plots were not treated. Between April and September, from 2007 to 2011, we deployed a total of 75,000 RTVs in Sherman traps and captured 3791 individual white-footed mice for an average deployment of ~20 oral RTVs per mouse. The average number of recaptures between highest and lowest for all grids/all years ranged from 4.3 to 8.6. In contrast to the controls, we observed a decline in NIP in all treated plots: NY1 2007–2011 (53, 51, 43, 34, 14%); NY2 2008–2011 (38, 25, 36, 35%); NY3 2009–2011 (47, 26, 25%) and NY4 2009–2011 (58, 26, 18%). Differences are statistically significant in the two plots that received RTV for the longest periods of time (NY1 2007–2011 χ^2, $p < 0.0001$ and NY2 2008–2011χ^2, $p = 0.0069$). In NY1, the overall difference between proportions between the first (2007) and the last (2011) year of the study was 45.70% (95% CI, 27.21–64.19%). In NY2, the overall difference between proportions between the first (2008) and the last year (2011) was 14.83% (95% CI, –2.63 to 32.29%). Accounting for declines in NIP validated by time, the difference between the highest (NY1, 45.70%) and lowest (NY2, 14.83%) reduction in NIP leads to a projection of 30.87% decrease in Lyme disease incidence in human populations over 4–5 years following sustained deployment of RTV in the field, as per Ginsberg's $P1 = 1 - (1 - k1)n$. Here, we report information that enables strategic implementation of a reservoir targeted vaccine by public health officials targeting hot zones for tick infection prevalence that would diminish the Lyme *Borrelia* threat from nature and could reduce Lyme disease cases by one third.

020

Efficacy of a single peridomestic application of acaricide to prevent Lyme and other tick-borne diseases

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Background: In the Northeastern USA, tick-borne diseases (TBDs) are a major public health concern and identifying effective prevention measures has been challenging. A single springtime application of acaricide has been shown to kill 95% of ticks in controlled studies. Although residential use of pesticides to control ticks is widespread in certain areas, the efficacy of residential pesticides to prevent human TBDs is unknown. Through a TickNET collaboration with the Centers for Disease Control and Prevention and Emerging Infections Programs in Connecticut, Maryland, and New York we sought to evaluate the efficacy of a single residential acaricide application to prevent TBDs.

Methods: In 2011 and 2012, we conducted a randomized, blinded, placebo-controlled trial in select areas of CT, MD, and NY. Participants were consenting adults from freestanding houses with >2 inhabitants and a yard. An enrollment survey was administered to measure select risk factors for TBDs and demographics. Households were randomly assigned to receive a single application of commercially available acaricide (bifenthrin) or placebo on their yard in spring, according to industry standards. Tick drags were conducted 2–3 weeks post-treatment on a 10% sample to verify treatment efficacy. Surveys regarding numbers of ticks attached/ crawling were administered at 1–4 months post-treatment. A final survey to capture self-reported TBDs was administered at 5 months. Self-reported TBDs were validated by medical record review.

Results: A total of 2646 participating households were enrolled over 2 years. Properties ranged from 1/2 to 5 acres in size. Post-treatment tick drags indicated a 75.8% reduction in ticks on treated vs. placebo properties in 2011 and a 48.4% reduction in 2012. However, the number of ticks encountered by participants was similar between the treated and placebo groups at 1–4 months post-treatment in 2011. Similarly, there was no difference in the number of cases of TBD. Analysis of 2012 data is pending.

Discussion: Although acaricide application was effective at reducing tick abundance in both years, data from 2011 indicate no reduction in tick encounters or disease. Reasons for this may include (1) periodomestic exposure is not a principle source of TBD risk; (2) the highest risk in the peridomestic environment may occur in untreated areas (e.g., gardens); or (3) the efficacy of acaricide treatments need to be significantly higher to affect TBD risk. These preliminary results highlight the importance of human studies for understanding the efficacy of tick-borne disease prevention efforts.

021

Potential for antibiotic creams to prevent Lyme disease spirochete transmission

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Although oral antibiotic formulations have been used to prophylactically prevent Lyme disease in clinical trials, the practice of prophylactically treating tick bite via oral doxycycline may not be widely practiced. As an alternative, the use of antibiotic creams placed directly onto the site where a tick was detected and removed may be attractive to residents of tick endemic regions. In the laboratory over the last decade, we have tried to develop an animal model to test optimal regimes for delivering antibiotics prophylactically. Using oral doxycycline, our results have been mixed varying from 23 to 74% efficacy; in addition, the oral doxycycline must be applied right at the time of tick removal. Accordingly, we tested cream formulations that could be applied to the site where ticks were allowed to attach and were removed from mice in a laboratory model. Doxycycline essentially showed no efficacy in this model. But, a 4% azithromycin cream was 100% effective blocking spirochete transmission in a single application when applied to the tick bite site. Moreover, the azithromycin cream was 100% effective when applied up to 72 h after tick removal. We hope to extend these observations that are confirmatory to success with azithromycin cream (albeit using different application regimes) in animal models in Europe.

022

Meta-analysis of the clinical presentation and epidemiological features of 128 *Borrelia miyamotoi* cases from Russia

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The borreliosis caused by *Borrelia miyamotoi* (Bmt) and transmitted by *Ixodes* ticks is a new human infection first described in Russia (Platonov et al., 2011). Recently, a few human cases of Bmt infection have been identified in the USA (Krause et al., 2012; Gugliotta et al., 2012). We summarize the clinical and epidemiologic features of Bmt infection in 128 cases in Russia in order to provide tools for a more effective search of the disease wherever Lyme disease is endemic. The search and laboratory confirmation of acute Bmt cases were carried out according to a previously published report (Platonov et al., 2011). Patients were enrolled after being bitten by *Ixodes persulcatus* ticks in Central Russia (Republic of Udmurtia and Sverdlovsk Region) in 2009–2012 between May 1 and July 30 that coincides with the peak period of tick activity. They developed flu-like symptoms between 11and 16 days after tick bite. Bmt DNA was found in their blood samples at admission. About 80% of the patients were adults (20–65 years) with a preponderance of males. Clinical symptoms consisted of fever (≥38°C), weakness, headache, and chills in nearly all the patients. Erythema migrans rashes were observed only in 4% of patients. More than that 60% of patients had signs of dysfunction in one or more organs, including the liver (jaundice with increased ALT and AST), kidney (proteinuria, elevated blood creatinine and urea levels), heart (myoglobin in blood, abnormal echocardiography), and lung (abnormal chest X-ray findings). Meningeal signs with normal cerebrospinal fluid were noted in 10% of patients. About half the patients had signs of hypercoagulation (mild initial signs of DIC). Most cases of Bmt infection were mild. Recurrent episodes of fever were observed in about 10% of cases with two or three episodes occurring before the start of antibiotic therapy. No serious sequelae of Bmt infection were noted in our patients, although follow up was limited. Serious neurologic sequelae and severe disease in pregnant women with adverse fetal outcome have been observed with other relapsing fever borreliosis. Additional studies will be needed to assess the global health burden of Bmt infection.

023

Long-term clinical outcome after Lyme neuroborreliosis in childhood

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Objectives: To determine long-term clinical outcome in children with confirmed Lyme neuroborreliosis and to evaluate persistent subjective symptoms compared to a control group.

Patients and Methods: After a median of 5 years, 84 children with confirmed Lyme neuroborreliosis underwent a neurological re-examination, including a questionnaire. Medical records were analyzed and a control group ($n = 84$) was included.

Results: The total recovery rate was 73% ($n = 61$). Objective neurological findings, defined as "definite sequelae," were found in 16 patients (19%). The majority of these children had persistent facial nerve palsy ($n = 11$), but other motor or sensory deficits occurred ($n = 5$). Neurological signs and/or symptoms defined as "possible sequelae" were found in another seven patients (8%), mainly of sensory character. Nonspecific subjective symptoms were reported by 35 patients (42%) and 32 controls (38%) (ns). Affected daily activities or school performance were reported to the same extent in both groups (23 vs 20%, ns).

Conclusion: The long-term clinical recovery rate was 73% in children with confirmed Lyme neuroborreliosis. Persistent facial nerve palsy occurred in 13% whereas other motor or sensory deficits were found in another 14%. Neurological deficits did not affect daily activities or school performance more often among patients than controls and should be considered as mild. Furthermore, nonspecific subjective symptoms such as headache, fatigue, memory, or concentration problems were reported as often among patients as controls, and should not be considered as sequelae after Lyme neuroborreliosis.

024

Elevated levels of IL-23 in a subset of patients with post-Lyme disease symptoms following erythema migrans

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Background: Erythema migrans (EM) and its associated symptoms typically resolve with 10–21 days of oral antibiotic therapy. However, about 10% of patients have subjective symptoms after antibiotic treatment, termed post-Lyme disease symptoms. Here, we investigated whether particular immune responses at the time of infection, or thereafter, correlate with the development of post-Lyme symptoms.

Methods: In previous European studies (*Am. J. Med.* 2010;123:79–86; *Clin. Infect. Dis.* 2012;55:343–350), 510 patients with EM were treated with 10–15 days of antibiotics and re-evaluated over 12 months. Of the 510 patients, 62 (12%) had post-Lyme symptoms. For this study, sera were available from 45 of the 62 patients. For comparison, sera were randomly selected from 41 patients whose symptoms resolved with antibiotic therapy. Serum levels of 23 cytokines and chemokines, representative of innate, and adaptive TH1, TH2, and TH17 immune responses, were assessed at each time point in both patient groups.

Results: Significant differences between groups were found for the TH1-associated chemokines, CXCL9 and CXCL10, and the TH17-associated cytokine, IL-23. Of the 86 patients, 85 had detectable levels of CXCL9, 84 of CXCL10, and 41 of IL-23. When stratified by culture results prior to treatment, the 39 patients with negative *Borrelia* cultures had significantly higher levels of CXCL9 and CXCL10, but lower levels of IL-23, than the 47 culture-positive patients, suggesting that TH1-type immune responses were more effective in spirochetal killing. When stratified according to post-Lyme symptoms, the levels of IL-23, but not CXCL9 or CXCL10, were significantly higher prior to therapy in patients who developed post-Lyme symptoms ($P = 0.04$), and these levels remained significantly elevated at subsequent time points. Moreover, of the 41 patients with detectable IL-23 levels, 25(61%) had post-Lyme symptoms, and all seven patients with levels >230 ng/ml had such symptoms. Because all patients became culture negative after antibiotics and because antibody responses to *Borrelia* VlsE C6 peptide declined similarly after treatment in patients with or without post-Lyme symptoms, persistent infection was not a likely cause of these symptoms. In contrast, antibody responses to human endothelial cell growth factor (ECGF), the first known autoantigen in Lyme disease, were more common in patients with post-Lyme symptoms ($P = 0.07$). Furthermore, antibody responses to ECGF, but not to VlsE C6 peptide, correlated directly with IL-23 levels ($R = 0.5$, $P = 0.02$).

Conclusion: Our findings indicate that a subset of patients has untoward TH17-type immune responses associated with the development of post-Lyme symptoms. These observations offer a new paradigm for the study of patients with post-Lyme disease symptoms.

025

Safety and immunogenicity of a novel multivalent OspA vaccine against Lyme borreliosis in healthy adults: a double–blind, randomized, dose–escalation phase I/II clinical trial

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Background*:* Lyme borreliosis (LB) is caused by *Borrelia burgdorferi* sensu stricto (s.s.) in the US and by multiple *Borrelia* species in Europe and Asia. No human vaccine is available.

Methods: The safety and immunogenicity of a multivalent vaccine containing protective epitopes from *Borrelia* outer surface protein (OspA) serotypes 1–6, formulated with or without aluminum hydroxide, was investigated in a study in Austria and Germany. Three hundred *Borrelia*-seronegative adults aged 18–70 years were equally randomized to receive three doses of either 30, 60, or 90 µg, 28 days apart, and a booster 9–12 months after the first immunization. In an extension of this study, 350 seronegative and seropositive subjects were enrolled to receive a similar dosing schedule of either 30 or 60 µg adjuvanted vaccine. Primary endpoints were the frequency and severity of reactions and the antibody responses to OspA serotypes 1–6, as determined by ELISA. The induction of biologically active antibodies was assessed by surface binding and bacterial killing assays. The study is registered with ClinicalTrials.gov, number NCT01504347.

Results: Adverse reactions were predominantly mild, and no vaccine-related serious adverse events were reported. The frequency of systemic reactions and the risk for moderate or severe systemic reactions was significantly lower for adjuvanted compared to non-adjuvanted formulations [risk ratio (RR) 0.54, $P < 0.001$; RR = 0.35, $P = 0.034$, respectively). Both 30 and 60 µg doses were well tolerated in seronegative and seropositive subjects. All doses and formulations induced substantial mean IgG antibody titers against OspA serotypes 1–6 after the primary (range 6944–17,321) and booster (19,056–32,824) immunizations. Post-booster antibody titers against all serotypes were significantly increased in adjuvanted compared to non-adjuvanted formulations ($p < 0{\cdot}0001$–$0{\cdot}0025$). In seronegative subjects, the 30 µg adjuvanted formulation induced the highest post-booster antibody titers [GMT range from 26,143 (95%CI 18,906–36,151) for OspA 2 to 42,381 (31,288–57,407) for OspA 5]. However, in seropositive subjects the 60 µg adjuvanted formulation induced significantly higher post-booster antibody titers [GMT range from 28,735 (95%CI 21,530–38,351) for OspA 1 to 42,381 (95%CI 32,991–54,442) for OspA 5] compared with the 30 µg adjuvanted formulation [GMT range from 12,653 (95%CI 8659–18,489) for OspA 1 to 17,485 (95%CI 12,470–2518) for OspA 5]. Vaccine-induced antibodies bound to and promoted the killing of *Borrelia* strains representing all major human pathogenic species.

Conclusions: The novel multivalent OspA vaccine is a promising candidate vaccine to prevent LB in the US and Europe, and possibly globally.

026

Epidemiology of Lyme borreliosis in Europe – what does trend analysis tell us?

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Lyme Borreliosis (LB), caused by spirochetes of the *Borrelia burgdorferi* s.l. complex, is the most common tick-borne infectious disease in the northern hemisphere mainly affecting skin, nervous system, or joints. Concise data on the epidemiology of LB in European countries are rare. This review aimed at providing current data on the incidence of LB in Europe. Data were collected by literature search in PubMed, grey literature such as national health registries, as well as personal contacts. Data on LB incidence of 32 European countries were obtained. Incidences among European countries do vary not only due to geographic location but also due to data acquisition (voluntary or mandatory reporting, studies, sentinels, varying enrolment criteria). Geographic distribution shows lowest incidences of LB in southwest Europe (Portugal $<0.7 \times 10^5$, 2007) and along the Mediterranean coast. Incidences increase northward (Sweden 69×1105, 1995) as well as from Western Europe (England and Wales 1.8×10^5, 2011) towards Central Europe (Switzerland 156×10^5, 2011). The highest incidence was reported in Slovenia (242×10^5, 2010). Data on Neuroborreliosis (NB) are available from 10 European countries with national incidences ranging from 0.6×10^1 in France to 11×10^5 in Sweden. Time series of incidences reveal major trends: countries with (I) ongoing upward trend such as Estonia, (II) an increase until 2006 followed by a stable phase like Germany, (III) undulating incidences such as Lithuania, and (IV) stable incidence such as Denmark or Norway. Data on the incidence of LB in Europe are heterogeneous and hard to compare due to the various approaches regarding data collection, enrolment criteria, case definitions, or the type and year of study. For the purpose of comparable data, well designed sentinel or prospective studies should be instituted. Although there is increase of LB in some countries over the past decade data do not support the hypothesis that LB is on a rise all over Europe.

027

Similar Lyme disease risk in contrasting host diversity settings

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Biodiversity's role in buffering against human infectious disease has been proposed as an ecosystem service widely applicable to a number of infectious diseases. The dilution effect hypothesizes that increased host diversity reduces pathogen transmission by reducing direct or indirect (through vectors) contact between infected and susceptible hosts. Biodiversity may also influence disease risk if pathogens can adapt, or specialize, to different host species. Lyme disease, caused by the bacterium *Borrelia burgdorferi* and transmitted by *Ixodes scapularis* ticks, is a common zoonotic disease in the northeastern United States. A wide range of taxa are competent hosts, but vary in their transmission efficiencies, with *Peromyscus leucopus* thought to be the most competent host. The dilution effect predicts that host communities dominated by *P. leucopus* represent the highest risk to humans, measured as the density of host-seeking *I. scapularis* nymphs. Human risk may also be increased in mouse dominated communities if there is positive selection for *B. burgdorferi* strains that have high persistence in mice (specialized to mouse hosts) and cause disseminating infection in humans. A comparative study of a host species-poor community dominated by *P. leucopus* (Block Island, RI) and a host species-rich community (Mansfield, CT) allowed us to evaluate support for the dilution effect and host specialization hypotheses. We examined three specific predictions of these hypotheses: (1) *B. burgdorferi* nymphal infection prevalence and density of infected nymphs is higher in the species-poor than species-rich community, (2) *B. burgdorferi* genotype diversity is lower in the species-poor than the species-rick community, and (3) "*P. leucopus*-adapted" strains are more prevalent in the species-poor community. We collected mammal-derived larvae and questing nymphs from 2010 and 2011 field seasons and sequenced *B. burgdorferi* DNA to estimate genotype diversity and richness. We found no differences in nymphal infection prevalence and density of infected nymphs between the two communities, providing no evidence for the dilution effect. We also found high amounts of genetic variation in the species-poor community, refuting the host specialization hypothesis. Additionally, proportions of disseminating *B. burgdorferi* strains found primarily in *P. leucopus*, were similar between species-poor and species-rich communities. We found little evidence for either the dilution effect or host specialization theories, suggesting that more research is needed to elucidate the complex factors driving *B. burgdorferi* infection and diversity patterns.

028

Predicting the rate of Lyme disease spread in Canada

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Identifying invading tick populations provides early warning for emerging tick-borne diseases that are spreading their geographic range. But how fast do tick-borne pathogens invade after ticks become established? Surveillance data for the tick *Ixodes scapularis* and the agent of Lyme disease *Borrelia burgdorferi* in southern Canada, where these species are invading, revealed a space-time cluster of ticks of low *B. burgdorferi* infection prevalence in southern Quebec signaling the location where tick populations became established beginning in 2004. The cluster disappeared in 2009 indicating a 5 year gap between tick and *B. burgdorferi* invasion. Simulations of a model of *I. scapularis* populations and *B. burgdorferi* transmission identified numbers of immigrating ticks rather than host density and diversity as key determinants of the speed pathogens invade after ticks become established. Greater numbers of immigrating infected nymphs would be expected in central compared to eastern Canada because in source populations in Midwestern USA, nymphal and larval ticks are active in spring when migratory birds can carry ticks north. In northeastern USA, tick populations that are sources for immigrating ticks for eastern Canada, nymphs but few larvae are active in spring. Consequently, we hypothesized that a 5 year gap would occur between tick and *B. burgdorferi* invasion in eastern Canada, but a much shorter gap would occur in central Canada. Consistent with this hypothesis, analysis of surveillance data revealed clusters of ticks with low infection prevalence of >5 years duration in locations in eastern Canada where *I. scapularis* is invading, but a non-significant cluster of only 3 years duration in regions of central Canada where *I. scapularis* is invading. We have identified the speed at which *B. burgdorferi* invades following *I. scapularis* invasion, and that synchrony of larval and nymphal tick activity in spring is a key factor determining the gap between tick and pathogen invasion. This has immediate application in interpreting imminence of Lyme disease risk when surveillance identifies emerging tick populations in Canada and general application in predicting of the speed of invasion of emerging tick-borne pathogens elsewhere.

029

The risk of acquiring *Borrelia* infection after a tick bite – a prospective follow-up study

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No previous study has, to our knowledge, investigated all the possible risk factors for the risk of developing Lyme borreliosis (LB), such as rate of *Borrelia* infections in tick population, frequency of human contacts with tick biotopes, length of tick exposure, *Borrelia* species, and number of spirochetes, individual susceptibility to infection, gender and age simultaneously. Therefore, a prospective epidemiological study, the tick-borne diseases (TBD) STING-study, was conducted, which aimed to investigate the clinical outcome and the serological responses in newly tick-bitten individuals and correlate the clinical data with findings in the ticks. The ticks that had bitten humans, blood samples, and questionnaires were collected from newly tick-bitten humans in Sweden and at the Åland Islands, Finland. The participants were followed-up after 3 months with collection of additional blood samples, ticks and questionnaires. The ticks and the feeding-time were analyzed with a USB-microscope, while the tick nucleic acid was analyzed for detection and quantification of both the *Borrelia* 5S–23S IGS and the 16S–23S IGS rRNA genes with two different PCR assays. The blood samples were screened for anti-*Borrelia*-specific antibodies with two different ELISA assays. Seroconversion, i.e., development of new antibodies, was defined as change from sero-negative to sero-positive, or a minimum two-fold increase in OD/index-values. The study subjects were considered to have a probable current *Borrelia* infection if their seroconversions were verified in a strip-immunoassay. Approximately one fourth of the 2154 ticks collected from 1568 study subjects harbored *Borrelia* spirochetes. However, only 51 study subjects (3.3%) showed seroconversion during the study period, and only 22 subjects (1.4%) were diagnosed with clinical LB. Self-experienced symptoms possibly associated with LB were reported by 15% of the study subjects, but only 45% attended health care due to the experienced symptoms. Thirty-eight percent of all ticks were removed within 24 h. The study subjects who seroconverted after bite by a *Borrelia*-infected tick removed their ticks later compared to non-seroconverted study subjects bitten by a *Borrelia*-infected ticks (median 62 and 31 h, respectively; $p < 0.001$). The number of spirochetes in the removed ticks did not differ between seroconverted and non-seroconverted subjects. In conclusion, the risk of developing LB after a bite by a *Borrelia*-infected tick is small, and does not seem to be correlated to the number of spirochetes in the tick. Asymptomatic infections are more common than diagnosed LB among those who become infected with *Borrelia* after a tick-bite from a *Borrelia*-infected tick.

030

Nymphal blacklegged ticks are not the vector of locally acquired Lyme disease in southeastern states: evidence from tick phenologies and human biting data

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In the northeastern and midwestern United States, the main vector of Lyme disease is the nymphal life stage of the blacklegged tick (*Ixodes scapularis*). One line of evidence for this conclusion is the strong association between disease onset and the timing of nymphal host-seeking, which in the north peaks from late spring to early summer. A second line of evidence is that the majority of *Borrelia*-infected ticks removed from humans in northern states by the Department of Defense's tick test program are nymphs. The incidence of Lyme disease cases reported from southeastern states is two orders of magnitude lower than in northern states, and peaks in summer. Unlike in the north, Lyme case onset dates in the Southeast correlate poorly with nymphal black-legged tick activity, which peaks earlier in the year as a consequence of warm spring temperatures. Furthermore, almost all blacklegged ticks removed from humans in the southeast are adults. We conclude that the summer peak in Lyme disease reports in southeastern states results from (i) northern-acquired cases diagnosed following summer travel to the south and (ii) mis-diagnosis of non-Lyme rashes and infections vectored by abundant, summer-active lone star ticks (*Amblyomma americanum*). Based on the human-biting data, we predict that the (low) numbers of locally acquired Lyme disease cases in southern states will have onset dates coinciding with the winter activity of adult blacklegged ticks rather than the activity of blacklegged nymphs. Analysis of recent Florida Lyme disease case data that distinguished local versus out-of-state infections supports this prediction.

031

OspC phyletic type influences mammalian host range

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OspC is an essential virulence factor of the Lyme disease spirochetes. While the precise function of OspC remains elusive, recent evidence suggests that it participates in interactions with host/tick derived ligands that are required for successful transmission. Phylogenetic analyses have revealed the existence of more than 30 genetically stable ospC phyletic types. It has been postulated that ospC diversity may have evolved to allow for expansion of the permissive host range of the Lyme disease spirochetes. Consistent with this, only a limited subset of ospC phyletic types have been demonstrated in isolates recovered from humans. To directly determine if ospC type influences infectivity, a panel of transgenic strains expressing different ospC genes (types A, E, F, H, M, Pko, Pbi, MOD-1, MOK-3a, and MOS-1b) in a *Borrelia burgdorferi* B31 genetic background were generated, characterized, and tested for their ability to establish infection in mice. Interestingly, the introduction of a type Pko ospC gene into the B31 background eliminated infectivity. This observation is consistent with earlier studies that demonstrated that strain Pko does not infect mice and instead preferentially infects other mammalian species. In addition, using a canine infectivity model, we sought to determine if the ospC genotype of strains that infect dogs differs from that reported for humans. *Ixodes scapularis* ticks collected in Rhode Island were fed on purpose bred dogs, tissue biopsies were collected, and the ospC genotype of the infecting strains determined. ospC genotypes rarely recovered from human Lyme disease patients from the same geographic region were found to predominate in canine infections. Collectively, the data presented here indicate a correlation between OspC type and the ability to establish infection in specific mammalian species.

032

Genomic characterization of lone star virus, a novel bunyavirus in the *Amblyomma americanum* tick

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Bunyaviridae is the largest family of viruses and infect a variety of plant, insect, and animal hosts. Recently, emerging tick-borne bunyaviruses have been discovered in association with acute febrile illness in humans, including the severe fever with thrombocytopenia syndrome virus (SFSTV) in China and heartland virus in the United States. Here, we used unbiased next-generation ("deep") sequencing to characterize the genome of lone star virus (LSV), an unclassified bunyavirus originally isolated from the lone star tick *Amblyomma americanum.* Cultures of LSV in a human (HeLa) and monkey (Vero) cell line demonstrated cytopathic effect within 72 h of incubation, and vacuolization was observed in infected Vero, but not HeLa, cells. In-depth analysis of ~7.6 million shotgun sequencing reads for viral sequences (~2 h on a 64-core computational server) and de novo assembly of the LSV genome (~6 h) was performed using an in-house developed rapid cloud computing-based pipeline for pathogen discovery in metagenomic data generated from clinical/environmental samples. The genome of LSV is a highly divergent phlebovirus in the family Bunyaviridae, sharing <31% amino acid identity with any other virus, and situated in a well-supported phylogenetic clade that includes the tick-borne SFSTV and Heartland viruses. Genomic characterization of LSV is an important step in the development of diagnostic tools to assess the risk of arbovirus transmission by *A. americanum*, a proven disease vector with an expanding geographic range and associated with southern tick-associated rash illness (STARI), which is clinically like erythema migrans but of unknown etiology. This study also underscores the utility of unbiased deep sequencing analyses in investigation of clinical samples for vector-borne pathogens of potential clinical and public health significance.

033

Anaplasma phagocytophilum AnkA and HDAC1 recruitment in epigenetic modulation of host gene transcription

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Anaplasma phagocytophilum (Aph) is *Rickettsia* of neutrophils transmitted by *Ixodes* spp. ticks and is the cause of human granulocytic anaplasmosis. Aph infection is associated with differential transcription of many neutrophil genes at a time when cellular function dramatically changes to promote bacterial survival and dissemination. In Aph infection, downregulation of many host defense genes occurs when histone 3 (H3) is deacetylated at their promoters. Cellular histone deacetylase (HDAC) expression and activity increase with Aph infection in HL-60 cells and overexpression promotes infection whereas pharmacologic inhibition and siRNA silencing of HDAC1 abrogate infection. Aph Ankyrin A (AnkA) is secreted via the T4SS, enters neutrophil nuclei, and binds to AT-rich DNA as at the CYBB promoter, where it silences transcription. AT-rich binding regions and repression of transcription are attributes of matrix attachment region binding proteins, like SATB1 that recruit HDAC1 for transcriptional silencing. We hypothesized that AnkA decreases transcription at CYBB by (i) endogenous HDAC activity; (ii) acting as a transcriptional repressor; and/or (iii) recruiting histone deacetylases. To address this hypothesis, recombinant AnkA (rAnkA) was tested for HDAC activity by enzymatic assay. We also used DNA pull-down of a biotinylated CYBB promoter and co-immunoprecipitation (co-IP) with AnkA mab to identify whether HDAC1 is associated with AnkA bound to DNA/chromatin in lysates from *A. phagocytophilum*-infected HL-60 cells or cells transfected and expressing AnkA. rAnkA lacks histone deacetylase activity *in vitro*. HDAC1 was bound to the CYBB promoter when AnkA was also bound, but not in uninfected cells that lack AnkA in DNA pull-down assays. Co-IP using AnkA mab also precipitated HDAC1, but not in cells lacking AnkA. The absence of endogenous HDAC in AnkA cannot explain CYBB H3 deacetylation. However, co-localization of HDAC1 and AnkA in DNA pull-down assays and the physical interaction of AnkA and HDAC1 in co-IP support AnkA recruitment of HDAC1, promoting H3 deacetylation, a closed chromatin configuration and gene silencing. While transcriptional changes often occur with intracellular pathogens that introduce secreted proteins into the host nucleus, this is the first example of a prokaryotic protein that directly binds host DNA and recruits chromatin modifying complexes to alter transcription for at least one locus that governs microbe survival. Whether AnkA or other Aph nuclear effectors contribute to an altered transcription program and function in neutrophils will be important to further investigate.

034

The lipid microdomains of *Borrelia*

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The presence of lipid microdomains that contain cholesterol-glycolipids on the outer membrane of the *Borrelia* (in both the Lyme disease and relapsing fever species) is important for structural, nutritional, and pathogenesis reasons. At the ultrastructural level, the lipoproteins OspA and OspB can be seen associated with the discrete microdomains on the outer membrane. Disruption or binding of the lipoprotein by antibodies results in a destabilization of the outer membrane. At the structural level, blind docking analyses have shown that the binding of both OspA and OspB to two of the cholesterol-glycolipids (microdomain forming lipids) could occur via the cleft within the exposed parts of the lipoproteins. It has been long known that cholesterol is required as a component of liquid medium (BSK) to grow *Borrelia* and this is provided as free cholesterol and as a complex with serum lipoproteins and in serum contaminants of albumin. This would suggest that *Borrelia* has the ability to incorporate free cholesterol directly from the medium. *Borrelia* can also acquire cholesterol directly from eukaryotic cell membranes following attachment in what appears to be lipid–lipid interactions. In a similar manner, *Borrelia* also can exchange its cholesterol glycolipids with cells either by direct attachment or by release of outer membrane vesicles charged with the lipids. This two-way exchange not only serves nutritional roles for the spirochetes, but also a means of transferring antigens to the surface of the cells. The cholesterol glycolipids are immunogenic and can elicit the development of antibodies that are cross-reactive with host molecules. Antibodies to the cholesterol glycolipids of *Borrelia* recognize surface molecules in eukaryotic cells and this cross reactivity could have an additive effect to the immunopathogenesis of Lyme disease. The exchange of cholesterol glycolipids and the response to the cholesterol glycolipids derived from the spirochetes on cells or matrix could also be an important aspect of the pathogenesis of the Borrelioses.

035

The *Borrelia burgdorferi* adhesin DbpA is bifunctional, binding to extracellular matrix to foster tissue colonization and to the host complement regulatory protein C4BP to promote bloodstream survival

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Borrelia burgdorferi sensu lato, the causative agent of Lyme disease, can infect the skin at the site of the tick bite and may subsequently spread via the bloodstream to the heart, nervous system, and joints, leading to multisystemic disease. Complement serves as a first line of immune defense in the bloodstream and pathogenic *Borreliae* produce surface proteins (e.g., Erp or Csp) that bind to host complement regulatory proteins such as Factor H to diminish complement activation and promote serum survival. *B. burgdorferi* produces two surface lipoproteins, decorin binding protein A (DbpA) and B (DbpB) that promote spirochetal binding to the extracellular matrix (ECM) components decorin and dermatan sulfate. A *B. burgdorferi* dbpBA mutant that is incapable of producing either DbpB or DbpA is unable to establish mammalian infection. In this study, we investigated the activities of DbpA that are required for colonization. Expression of wildtype DbpA, but not DbpA C11, which lacks the C-terminal 11 amino acids of DbpA and is incapable to bind to either decorin or dermatan sulfate, promoted stable murine colonization by *B. burgdorferi* dbpBA, indicating that the binding activity of DbpA is critical for colonization. Interestingly, unlike the parental *B. burgdorferi* dbpBA mutant, derivatives that ectopically produce either DbpA or DbpA ΔC11 were detected in the bloodstream at 3 days post-infection and were resistant to human serum *in vitro*. Further, pull-down assays of human serum and quantitative binding analyses using recombinant DbpA revealed that DbpA recognizes the complement regulatory protein C4-binding protein (C4BP) with high affinity (KD = 0.82 ± 0.03 μM). These findings suggest that DbpA is a bifunctional protein that binds ECM to promote tissue colonization and C4BP to protect the spirochete from immune clearance at early stages of infection.

036

Borrelia burgdorferi infection in *Ixodes ricinus* ticks increases their chances to find a host

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An increasing number of studies suggest that vector-borne parasites are able to alter phenotypic traits in their arthropod vectors in a way that it would enhance microorganism transmission. Ticks transmit pathogens causing human health concerns throughout the world, in particular *Borrelia burgdorferi sensu lato* (s.l.), the agent of Lyme disease. The vector of *B. burgdorferi* s.l. in Europe, *Ixodes ricinus*, is very sensitive to desiccation. During questing for hosts on vegetation, ticks experience desiccating conditions so that they have to regularly move to the litter zone to rehydrate. Moving down and up, vegetation reduces tick energy reserves, which is detrimental for tick survival. In addition, rehydration periods in the litter layer decrease the chances to find a host by reducing questing time. Therefore, a better resistance to desiccation would increase tick lifespan as well as the chances to find a host and subsequently transmit pathogens. This led us to investigate whether *Borrelia* infection increases *I. ricinus* survival under hot and dry conditions and decreases their need for humidity. Results showed (1) that under desiccating conditions *Borrelia*-infected nymphs and adults survived better (43 and 50% survival, respectively) than their uninfected counterparts (28 and 38% survival, respectively) and (2) that *Borrelia*-infected ticks moved less (36%) to an environment favorable for maintaining water balance than uninfected individuals (51%). This corroborated our hypothesis suggesting higher tolerance to desiccation in *I. ricinus* ticks that are infected by *B. burgdorferi* spirochetes. We then investigated whether the higher tolerance to desiccation in infected ticks was related to higher energy reserves. We observed that, in fact, *Borrelia* infection in *I. ricinus* nymphs was associated with higher lipid content (reflecting tick energy reserves). For a given size *Borrelia*-infected ticks harbored 12% more fat than uninfected individuals. Hence, *I. ricinus* ticks benefit from *Borrelia* infection by an increased lifespan (more fat and more resistance to desiccation) and by an increased questing period (less need to move to the litter zone). In fine, *Borrelia* infection enhances tick chances to find a host and to subsequently transmit the pathogen. Although the underlying mechanisms leading to our results remain to be fully understood, our observations contribute significantly to the acknowledgement of the influence of *B. burgdorferi* s.l. spirochetes on phenotypic traits of their tick vector *I. ricinus* optimizing their transmission to the next host.

037

Transmission of dynamics of *Borrelia turicatae*

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Relapsing fever spirochetes are a globally neglected pathogen and very little is known regarding the events involved with vector colonization and transmission. Our lab studies the *Borrelia turicatae–Ornithodoros turicata* model of relapsing fever spirochetes, and a unique characteristic of pathogen–host interactions when compared to other tick-borne disease is the rapidity of transmission. Depending on the developmental stage, *O. turicata* will feed to repletion within 5–60 min. Given the duration of the bloodmeal, relapsing fever spirochetes have evolved mechanisms to establish long-term infections within the salivary glands and are consequently transmitted in a relatively short period of time. With very little known regarding salivary gland colonization by *B. turicatae*, we have commenced studies to quantify spirochete densities within the tissues and to assess the rapidity of transmission. Time trials were performed where we determined transmission frequencies between ticks that were allowed to attach onto mice for 10 s when compared to ticks that were allowed to engorge (a process that that was completed within 5–10 min). Our findings characterize spirochete densities within the salivary glands and indicate that *B. turicatae* is transmitted within seconds of attachment.

038

Inability to utilize certain carbohydrates results in loss of fitness for *Borrelia burgdorferi* in the enzootic cycle

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Background: During the life cycle of *Borrelia burgdorferi*, nutrient availability is an important factor that contributes to the modulation of gene expression for survival in mammals and the tick vector. Glycerol 3-phosphate dehydrogenase (GlpD) is responsible for channeling glycerol utilization to the glycolytic pathway. GlpD mutants were able to complete the tick–mouse cycle, but showed decreased fitness in unfed nymphs and delayed transmission to naïve mice. We aimed to identify the alternate carbohydrate that the GlpD mutant utilizes to survive the tick–mammal cycle in the absence of glycerol utilization. A candidate source is chitobiose, the monomer of chitin, which is found in the tick cuticle. ChbC is a component of the chitobiose transporter required for uptake of chitobiose.

Methods: A glpD-chbC double mutant was constructed by disrupting the glpD gene in a chbC mutant strain. The double mutant strain's ability to survive and be transmitted to naïve mice was assessed and compared to wild type and the respective single mutant strains during the tick–mouse cycle.

Results: The glpD-chbC double mutant and single mutants were infectious in needle inoculated mice and had spirochete densities in fed larvae comparable to wild type. The double mutant had significantly lower spirochete load in flat nymphs relative to wild type ($p < 0.05$). In fed nymphs, the chbC mutant had significantly lower spirochete loads compared to wild type ($p < 0.05$). Additionally, the chbC and glpD-chbC mutant strains both required a longer duration of tick attachment for spirochetal transmission to naïve mice. Interestingly, whereas a GlpD mutant could successfully infect all mice after 72 h of tick feeding, only one of eight mice could be infected by ticks containing glpD-chbC mutants under similar conditions.

Conclusion: The data suggest that glpD mutants are not utilizing chitobiose to survive in the nutrient poor flat nymph. Currently, the identity of the alternative carbohydrate source is not known. The simultaneous disruption of both glpD and chbC appears to have a more severe phenotype for transmission than disruption of either gene alone. Additionally, the data suggest that utilization of chitobiose plays a role in the feeding nymph and is required for subsequent transmission of the spirochete to the mammalian host.

040

Microrna MIR-146A is a critical suppressor of arthritis in the mouse model of Lyme disease

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Lyme disease is caused by infection with *Borrelia burgdorferi*, a tick-borne spirochete, is endemic to temperate regions in the northern hemisphere, and is the most common vector-borne disease in the United States. Often, infection leads to acute arthritis in humans. Our lab uses the mouse model of Lyme disease to study the molecular mechanisms of arthritis development. MicroRNAs (miRNAs) are small, non-coding RNAs that are involved in posttranscriptional gene regulation. miRNAs act as inhibitors of translation, are capable of targeting many different genes, and are important in regulating many cellular processes, including immune response to pathogens. Abnormal miRNA expression and function has been linked in humans to a variety of immune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). However, the importance of miRNAs in bacterial infections and inflammation is just beginning to be appreciated, and no studies have been published on how miRNAs regulate immune response to *Borrelia* infection. To identify miRNAs important to suppression of Lyme arthritis, we performed an unbiased screen of miRNA expression during infection with *B. burgdorferi* in the arthritis-susceptible C3H and IL10–/– (B6) mouse strains, as well as in the arthritis-resistant B6 mouse strain. This screen identified several candidate miRNAs differentially regulated during host response to this pathogen. These included many that have been identified previously as being important regulators of immune function. Among these, miR-146a was highly up-regulated in all three strains. Previous studies have shown that miR-146a suppresses NF-kB signaling, and we and others have demonstrated that TLR2/MyD88 dependent activation of NF-kB is a major regulator of immune response in mice and humans, but determining its role in arthritis development has remained elusive. We hypothesized that this miRNA was acting as an immune suppressor during infection with *B. burgdorferi*. Furthermore, we predicted that arthritis-resistant B6 mice lacking miR-146a would harbor fewer bacteria and have more severe arthritis. We therefore infected age-matched miR-146–/– (B6) mice with *B. burgdorferi*, along with WT B6 controls. As predicted, miR-146a–/– mice developed significantly more severe arthritis at four weeks post-infection. Infected joints also contained significantly fewer bacteria in infected joints, as measured by B. burgdorferi-specific16S-rRNA. These data showed that increased arthritis observed in miR-146a–/– mice is due to a defect in host immune regulation, rather than host defense. We used fluorescence-activated cell sorting (FACS) followed by qRT-PCR to identify cellular sources of miR-146a in infected joint tissue. We found that miR-146a was highly enriched in both the myeloid and lymphoid cell fractions, but was not detectible in endothelial and synovial fibroblast-enriched cell fractions. Additionally joints from infected miR-146a–/– mice contained higher numbers of myeloid cells at two weeks post-infection, suggesting that these are important effecter cells during arthritis development. Consistent with NF-kB dysregulation, mice had significantly higher levels of NF-kB-induced cytokines, such as IL6, and elevated transcript levels of arthritis-promoting chemokines, such as CXCL1 and CXCL10. These data show that miR-146a is critical in regulating NF-kB signaling during infection, and is the first known model to directly link NF-kB dysregulation to arthritis development. This novel model provides the field a much-needed system for elucidating the role of NF-kB in Lyme Arthritis development, and provides a valuable therapeutic target for individuals suffering from Lyme Arthritis and other related immune disorders. Finally, this study highlights the critical role of miRNAs in regulating host response to *B. burgdorferi* infection and inflammation.

041

Human TLR10 is a pattern recognition receptor with a suppressive function

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Toll-like receptor (TLR) 10 is the only pattern recognition receptor without known ligand specificity and biological function. TLR10 is a part of the genetic locus that encompasses TLR1, TLR2, and TLR6. TLR2 is biologically active as a homodimer and as heterodimers formed with TLR1 or TLR6. Since TLR10 also dimerizes with TLR2, we reasoned that TLR10 may also exert its biological function as a heterodimer with TLR2. When TLR10 on human peripheral blood mononuclear cells (PBMC) was blocked with a specific neutralizing antibody, we found increased cytokine production after stimulation with Pam3Cys or *Borrelia burgdorferi*. These data point to an inhibitory role for TLR10 in conjunction with TLR2. This was further established using monocyte-derived dendritic cells exposed to the same antibody. We found upregulation of sPam3Cys-induced cytokine responses when the antibody was added. Next, we transfected HEK293 cells, either with TLR2 alone or with TLR2 and TLR 10. When cells co-transfected with TLR2 and TLR10 were exposed to TLR2 ligands (Pam3Cys or *Borrelia burgdorferi*), cytokine production was inhibited compared to cells transfected with TLR2 alone. This effect was found to be specific for TLR2, as TLR5-induced cytokine production was not affected by co-transfection with TLR5 and TLR10. Further evidence for the inhibitory role of TLR10 was obtained with RNA silencing of TLR10 in PBMC and in monocyte-derived macrophages; here too, we found enhanced cytokine production when these cells were exposed to TLR ligands. The inhibitory effect of TLR10 on cytokine production is mediated by the p38 MAPK-pathway. Finally, individuals bearing TLR10 polymorphisms display an increased capacity to produce cytokines upon stimulation with TLR2, but not TLR4 ligands in a gene-dose-dependent manner. These results demonstrate that the biological function of the orphan receptor TLR10 is to downregulate TLR2-mediated cytokine responses and establish TLR10 as the first TLR with a suppressive function.

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Human TLR8 is activated upon recognition of *Borrelia burgdorferi* RNA in the phagosome of human monocytes

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Phagocytosed *Borrelia burgdorferi* (Bb) induces innate immune signals in human monocytes which include transcription of IFN-β. We have previously reported that in response to phagocytosed Bb, TLR8 co-operates with TLR2 in the induction of NF-κB mediated cytokines, while TLR8 is self-amplifying and solely responsible for induction of IFN-β, through IRF-7. Aiming to dissect these phagosomal signaling processes and to identify the ligands, which trigger these responses in humans, we first utilized HEK.293 cells stably transfected with human TLR2, TLR-7, or TLR-8, along with an NFk-B reporter. We show that borrelial lysates and lipidated ipoproteins are internalized and activate human TLR2 in HEK.293 cells. We also demonstrate that Bb RNA activates human TLR8. On the other hand, TLR8 was not activated by Bb DNA, nor by Vaccinia virus DNA, which like Bb is A-T rich. Neither RNA nor DNA induced activation of human TLR7 in HEK.293 cells. We then stimulated highly purified human monocytes with live Bb (MOI 10:1) and equivalent amounts of Bb DNA or RNA. We observed that delivery of Bb RNA and DNA into the phagosomes elicits transcription of IFN-β through IRF7. Using confocal microscopy, we show that TLR8 colocalizes with internalized Bb-RNA bound to its fluorescent delivery vehicle (PEI) and in both early (EEA1) and late endosomes (LAMP-1). RNA staining of internalized live Bb confirms that once internalized the bacterium is confined to the phagosome. Utilizing fluorescent dextran particles and GFP-Bb as well as ethynyluridine-stained Bb RNA, we provide evidence that once phagocytosed, neither Bb nor its RNA leak into the cytosol. Initially associated with recognition of ssRNA of viral origin, our findings demonstrate that phagosomal TLR8 activation occurs upon recognition of bacterial RNA. We conclude that Bb RNA constitutes the TLR8 ligand responsible for eliciting type I IFN signals in human monocytes and these responses take place solely from within the phagosome of human monocytes.

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Borrelia bavariensis sp. nov. resist complement-mediated killing by a novel immune evasion mechanism

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Spirochetes of the *Borrelia burgdorferi sensu lato* complex differ in their resistance to human complement. So far, resistance directly correlates with the ability of *Borreliae* to bind host-derived complement regulators of the alternative pathway (AP), factor H, and factor H-like protein-1 via genetically distinct complement regulator-acquiring surface proteins (CRASPs). In the present study, we investigated *Borrelia bavariensis* to gain deeper insight into the molecular mechanism of immune evasion of this neuroinvasive *Borrelia* species. The contribution of complement regulators of the AP, classical pathway (CP), and terminal pathway in facilitating complement resistance of *B. bavariensis* was examined by employing serum adsorption assays. Interestingly, none of the serum-resistant *B. bavariensis* isolates tested were able to bind complement regulators in detectable amounts. Additionally, two CspA orthologous proteins of *B. bavariensis,* BGA66 and BGA71, were analyzed regarding their intrinsic regulatory activity of the CP and AP by performing hemolytic assays as well as their capacity to inhibit formation of the membrane attack complex. These functional analyses revealed that BGA71 was able to inhibit activation of the CP, while BGA66 blocked activation of both, AP and CP. To examine the mode of action of BGA66 and BGA71 *in vivo*, a serum-sensitive *B. garinii* strain lacking all CRASPs was transformed with individual shuttle vectors harboring the entire BGA66 or BGA71 encoding genes. Employing growth inhibition assays, survival of transformants in non-immune human serum (NHS) was analyzed. Spirochetes producing either BGA66 or BGA71 on their surface were able to survive in the presence of NHS, indicating that both proteins exhibit complement inhibitory activity. Our data reveal a novel immune evasion strategy of *B. bavariensis* that is independent from binding of complement regulators. Moreover, the molecular mechanism involves direct interaction of BGA66 and BGA71 with complement components, resulting in complete protection of *B. bavariensis* from complement-mediated killing.

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The role of DBPA and B adhesins of *Borrelia burgdorferi sensu lato* in the adhesion of *Borrelia* to endothelial cells

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Objectives: Lyme borreliosis (LB) is an infectious disease caused by the *Borrelia burgdorferi sensu lato* spirochetes. Systemic dissemination of LB from the initial infection focus on the various target organs is thought to occur via the hematogenous route. However, the molecular mechanisms mediating the interaction between the spirochetes and the vascular endothelial cells remain incompletely understood. There are three major *Borrelia* genospecies, *Borrelia burgdorferi sensu stricto* (Bbss), *Borrelia garinii* (Bg) and *Borrelia afzelii* (Ba) causing disease in humans. Decorin binding proteins (DbpA and B; Dbps) of the three genospecies differ in the amino acid sequence, and our earlier results show that they, indeed, have different binding properties to decorin potentially leading to differences in dissemination and tissue tropism of the bacteria. Interestingly, we have shown that under stationary incubation, DbpA of Bg showed a weak adherence to decorin, whereas under flow the same protein bound to decorin with high affinity. The present study aims at clarifying the role DbpA and B adhesins of different genospecies in the adherence of the spirochetes to human endothelial cells.

Methods: A DbpA and B deficient knock out strain was modified to express dbpAB operon of Bg, Ba or Bbss. HUVECs isolated from umbilical cords were cultured in plastic chamber slides. Fluorescently labeled bacteria were allowed to adhere to the cells. The interaction of *Borrelia* with endothelial cells was recorded and quantified with a confocal microscope.

Results: Our results showed that adherence of *Borrelia* to HUVECs under stationary incubation was DbpA and/or B dependent. The strains expressing DbpA and B of Bg and Bbss exhibited clear adhesion to the cells, while the binding of the strain with Dbps of Ba was low. As control, the DbpA and B deficient knock out strain did not bind to the HUVECs.

Conclusion: Dbps of Bbss, Bg, and Ba contribute differently to HUVEC adhesion of *Borrelia* under stationary incubation. Experiments addressing adhesion of the strains to HUVECs under flow are on-going. We are also working on the identification of the receptor molecules of endothelial cells.

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Lipoprotein succession in *Borrelia burgdorferi*: similar but distinct roles for OspC and VlsE at different stages of mammalian infection

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The spirochete *Borrelia burgdorferi* alternates between tick and mammalian hosts, requiring major changes in gene expression and protein production to adapt to these diverse niches. Among the adaptations observed is a shift among the major outer surface lipoproteins OspA, OspC, and VlsE at different stages of the infectious cycle. We hypothesize that this series of outer surface proteins carry out a basic but essential function, and that OspC and VlsE fulfill this requirement during early and persistent stages of mammalian infection, respectively. Previous work by other investigators suggested that several *B. burgdorferi* outer-surface lipoproteins, including OspA and VlsE, could substitute for OspC at the initial stage of mouse infection, when OspC is transiently but absolutely required. In this study, we assessed whether vlsE and ospA could restore infectivity to a *B. burgdorferi* ospC mutant and found that neither protein effectively compensated for the absence of OspC during early infection. In contrast, we determined that the requirement for OspC extended throughout infection if VlsE was not available. Such OspC-producing (VlsE-deficient) spirochetes can persist in SCID mice, where OspC is not a target of an acquired immune response. Together these results indicate that OspC can substitute for VlsE when the capacity for antigenic variation is irrelevant, but these two abundant lipoproteins are most likely optimized for their related but specific roles during early and persistent infection of mammals by *B. burgdorferi.*

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Role of the DNA mismatch repair protein mutS in VlsE recombination and infectivity of *Borrelia burgdorferi*

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Antigenic variation is a common mechanism of immune evasion, a process by which organisms change their antigenic proteins and thereby gain a selective advantage over individuals that retain parental antigenic determinants, resulting long term of persistent infection. The 35 kDa surface-exposed lipoprotein VlsE undergoes antigenic variation during *Borrelia burgdorferi* infection in mammalian hosts and humans, and is believed to play a major role in the pathogenesis of *B. burgdorferi* and infection. Little is known about the role of biological factor in VlsE recombination. Previous studies indicated that inactivation of ruvA and ruvB genes caused reduced infectivity phenotype and greatly diminished rate of vlsE recombination in *B. burgdorferi*. To assess the roles of potential trans-acting factors in vlsE recombination, we screened transposon mutants of genes known to be involved in DNA replication, recombination, and repair for their effects on infectivity and vlsE recombination including those in BB0797 (mutS), BB0098 (mutS-II), and BB0211 (mutL). Mutation in gene mutS exhibited an intermediate infectivity phenotype in C3H/HeN mice, a mutant in mutS-II was noninfectious in both immunocompetent and severe combined immunodeficiency (SCID) mice. PCR-RFLP results indicated that mutS had reduced vlsE RFLP variability. Analysis of 272 vlsE sequences from *B. burgdorferi* clones indicated that the mutS mutant exhibited a reduced rate of vlsE recombination. The mutS mutant had delayed clearance of parental sequence relative to the parental strain (5A18NP1) and fewer unique vlsE sequences, the mutS mutant was full infectious inC3H/scid mice, indicating that failure to evade the immune response is the basis for compromised infectivity of mutS mutant in immunocompetent. These data provide evidence that MutS is another protein important in vlsE recombination, antigenic variation, and infectivity in *B. burgdorferi*.

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Defining the compartmentalization of the *Borrelia burgdorferi* B31 lipoproteome

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The current list of known virulence factors of the Lyme disease agent *Borrelia burgdorferi* is dominated by lipoproteins, but the compartmentalization of this class of peripherally anchored membrane proteins within the spirochetal has not been explored comprehensively. To do so, we set out to clone all predicted lipoprotein genes of the *Borrelia burgdorferi sensu stricto* type strain B31 into a pBSV2-derived expression plasmid that provided a 5′ flaB promoter for constitutive expression and a 3′ hexahistidine tag for protein detection. The resulting plasmid library was then used to transform the B31 clone B31e2, and an established protocol of proteolytic shaving, membrane fractionation, and Western immunoblotting were used to localize the recombinantly expressed lipoproteins. Seventy-seven lipoproteins were localized to the bacterial surface, while 29 were localized to the periplasm. Six of the latter were detected in the inner membrane, and six in the periplasmic leaflet of the outer membrane. Expression of 13 lipoproteins could not be detected, suggesting either C-terminal processing or protein instability; lack of a hexahistidine-tag Western immunoblot signals in a mutant lacking the C-terminal protease CtpA support the latter. Five lipoprotein genes could not be cloned. Ultimately, the results of this study will be crucial in defining the spirochetal cell envelope as well as canonical lipoprotein localization determinants. Furthermore, they may aid in the identification and characterization of new vaccine candidates.

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Use of nonelectrolytes reveals the channel size and oligomeric structure of the *Borrelia burgdorferi* P66 porin

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P66 is an outer membrane protein found in the Lyme disease spirochete *Borrelia burgdorferi.* The protein forms pores in artificial lipid bilayers with an extremely high single channel conductance of 11 nS in 1 M KCl. In this study, single-channel conductance experiments in the presence of nonelectrolytes of known hydrodynamic radii were performed. This was done to investigate the actual diameter of the P66 channel. Nonelectrolytes with a hydrodynamic radii equal or below 0.34 nm could pass through the pore. Neutral molecules with larger radii could only partially fill the channel or were not able to enter it. From these results, the P66 channel was predicted to be ≤1.9 nm with a 0.8 nm diameter constriction site. When analyzing the complex formed by P66 with PEG 400, PEG 600 or maltohexaose blockage of one P66 single-channel conductance unit was found to occur in about eight subconductance states. This indicates that the P66 channel could be an oligomer of around eight individual channels. BlueNative PAGE and immunoblot analysis confirmed this possible organization of P66 and revealed a protein complex of ~460 kDa. A second dimension SDS PAGE showed that P66 is the only component of this pore-forming protein complex. Ongoing studies aim to obtain the crystal structure of the P66 protein. Large amount of P66 protein purified from a P66 over expressing B31 A3 *B. burgdorferi* strain has been obtained in milligram quantity, which will be further used for structural and biochemical analysis.

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Borrelia burgdorferi oxidative stress regulator BosR directly represses lipoproteins primarily expressed in the tick during mammalian infection

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Differential gene expression is a key strategy adopted by the Lyme disease spirochete, *Borrelia burgdorferi*, for adaptation and survival in the mammalian host and the tick vector. Outer surface protein A (OspA) is expressed during tick colonization and repressed in the mammal. The *Borrelia* oxidative stress regulator (BosR) is a DNA-binding protein belonging to the ferric uptake regulator (Fur) family. Here, we show that BosR is required for OspA repression in the mammal. Furthermore, BosR directly binds to the ospA promoter through recognition of palindromic sequences homologous to those recognized by other Fur homologues, but with a less stringent requirement for the spacer length. A total of 156 *B. burgdorferi* genes have been identified to have one or more putative BosR-binding sites in their promoter region. Remarkably, 12 of the 19 genes which were previously identified in a genome-wide microarray study to be >fivefold repressed in the mammal are among this putative BosR regulon. Footprint analysis using *B. burgdorferi* genomic DNA showed that promoter regions containing putative BosR-binding sites were protected by BosR from DNase I digestion. These data suggest that BosR directly represses transcription of many genes, which are down-regulated in the mammal.

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Persistence of *Borrelia burgdorferi*, the Lyme disease spirochete, within its arthropod vector involves three distinct genetic programs

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Following acquisition from an infected mammalian host during the larval blood meal, *Borrelia burgdorferi* (Bb) must adhere to rapidly differentiating midgut epithelial cells, resist deleterious substances within the lumen, and alter its metabolic machinery to utilize alternative carbon sources. During transmission, spirochetes presumably must cope with these same bloodmeal-associated stressors, while traversing the midgut and salivary glands in order to gain access to the mammalian host. A major conundrum in Lyme disease research is how acquisition and transmission can elicit ostensibly similar protective/physiologic adaptations, yet evoke such markedly different spirochete behaviors. Spirochetes lacking the alternative σ factor RpoS are impaired in network formation, the initial non-motile of phase of biphasic dissemination. Using site-directed and Tn-mediated mutagenesis, we now have identified nine RpoS-upregulated genes (cdr,mcp45, a04, a24, a33, a34, a64, a72, and b09) involved in infection via tick bite, results which collectively support our conceptualization of RpoS-dependent gene expression as a "Go" signal for tick-to-mammal transmission. In contrast, spirochetes within acquiring ticks remain associated predominantly with the luminal surfaces of the midgut and, importantly, do not form networks. Bb within feeding larvae are in an RpoS-OFF state, thereby allowing for de-repression of ospA, the prototypical tick-phase borrelial gene. OspA-deficient Bb survive the larval blood meal, but do not persist through the molt, enabling us to conclude that the RpoS-OFF state contributes to the induction of a tick-adaptive "Stay" signal. Spirochetes lacking the Hk1/Rrp1 two-component system (TCS), which signals via synthesis of c-di-GMP, are destroyed within the midguts of feeding larvae. Structural modeling of the Hk1 periplasmic sensor points to tick- and/or host-derived neurotransmitters as potential activating ligands. Side-by-side comparison of Δhk1, Δrrp1, and Δglp Bb revealed that Hk1/Rrp1-dependent gene products aside from those involved in glycerol uptake and utilization are required to counter noxious substances associated with tick-feeding; we postulate that this occurs via remodeling of the borrelial cell envelope. The requirement for c-di-GMP signaling within feeding nymphs demonstrates that the blood meal-associated stressors that Bb encounters during acquisition also are present during transmission. In summary, the tick phase of Bb's enzootic cycle appears to involve three distinct genetic programs: RpoS-ON, RpoS-OFF, and Hk1/Rrp1-ON, which provide Go, Stay, and blood meal-survival signals, respectively.

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Bpur, a novel *Borrelia burgdorferi* nucleic acid-binding protein, has global impacts on the proteome and Lyme spirochete infectivity

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Lyme disease spirochete, *Borrelia burgdorferi*, persists in nature through infectious cycles between vertebrate hosts and tick vectors. Precise regulation of bacterial gene and protein expression is critical for all borrelial infection processes. We have identified and biophysically characterized a novel *B. burgdorferi* nucleic acid-binding protein, which we named Bpur. Intriguingly, Bpur is highly similar in both sequence and structure to the PUR-domain proteins of humans and other eukaryotes, which are crucial cellular regulatory factors. ChIP-seq and RIP-seq demonstrate that *B. burgdorferi* Bpur binds to both RNA and DNA in live bacteria. We produced a mutant of *B. burgdorferi* in which Bpur is locked into an "ON" state and, using next-generation RNA-sequencing and proteomic analyses, observed significant changes in the levels of over 50 mRNAs and proteins. In addition, the Bpur-ON mutant is significantly impaired in ability to infect mammals. Given these effects, it is not surprising that Bpur expression is tightly regulated, in part, by a self-regulatory mechanism at the post-transcriptional level. Taken together, these features make Bpur an attractive target for development of novel anti-borrelial therapies. Furthermore, since most eukaryotes produce PUR domain proteins, these results provide not only new information on gene regulation in the Lyme spirochete, but on critical aspects of protein–nucleic acid interactions spanning all forms of life.

A001 (EC001)

Gradients in blacklegged tick abundance and infection prevalence with *Borrelia burgdorferi* correlated with distribution of Lyme borreliosis throughout the eastern U.S.A.

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In the eastern U.S., a striking contrast exists between the distributions of Lyme borreliosis and its vector tick. Whereas most human cases occur in the North, the blacklegged tick is found throughout the region. Ticks infected with the agent of Lyme borreliosis are, however, rarely found in the South. Several hypotheses have been proposed to explain the North–South gradients in Lyme borreliosis and infected ticks. Over the last 3 years, our research team has worked to test these hypotheses by integrating field and laboratory data with modeling studies. We obtained ecological data at nine replicate field sites throughout eastern U.S. and tested three major classes of hypotheses: (1) vertebrate host community composition effects on maintenance cycles of *Borrelia burgdorferi*; (2) climate factors on tick abundance, seasonal activity, and questing behavior; and (3) tick genetic factors affecting the same. Here, I will present the results of geographic variation in tick abundance and infection prevalence to test the hypothesis that blacklegged tick populations are too scarce in the South to maintain *B. burgdorferi*. Blacklegged ticks were present at all our sites, despite great variation in habitat type ranging from swampy lowlands to mixed uplands. Peak questing adult abundance varied markedly, from <10 ticks to ~90 ticks per 1000 m^2. The density of questing adults was highest in the North, but the abundance at our Florida site ranked higher than our Massachusetts and New Jersey sites. Nymphs were dragged at all sites, but rarely from southern sites. Questing adults were present everywhere, so these results confirm strongly the hypothesis that nymphs in the South rarely host-seek above the leaf litter, and so pose little risk of transmitting pathogens to humans. This would be the case even if they were infected with *B. burgdorferi*; our assays also confirm that adult infection prevalence (range 0–40%) is lowest in the South, although 3% of questing adults at our Florida site were infected. This widely distributed survey for both adult and nymphal ticks shows that: (1) blacklegged ticks are present throughout the South; (2) their densities in the South are generally much lower than in the North, although certain habitats support dense populations; and (3) infection with *B. burgdorferi* is far more prevalent in the north (even though some southern populations are more abundant that some highly-infected northern populations). Additional factors – including vertebrate host composition, host-preference, and host-seeking phenology – may contribute to *B. burgdorferi* maintenance in blacklegged ticks.

A002 (EC003)

Comparison of phenology and pathogen infection rates of *Ixodes scapularis* removed from soldiers at Camp Ripley, MN; Ft. McCoy, WI; and Ft. Indiantown Gap, PA; 1997–2012

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Two different observations sparked this comparison among *Ixodes scapularis* removed from Soldiers and submitted to the Department of Defense Human Tick Test Kit Program. First, the adult life stage of *I. scapularis* was more frequently received from Camp Ripley, MN, in the summer months (June and July) than from other U.S. installations. Second, the novel human pathogen, Ehrlichia species "Wisconsin" or "EML" was detected in *I. scapularis* removed from Soldiers only from MN and WI, but not from any other installations within the range of the tick. We compared the phenology and pathogen infection rates of *I. scapularis* removed from Soldiers at Camp Ripley, MN; Ft. McCoy, WI; and Ft. Indiantown Gap, PA. Many variables among the installations are very similar. The three facilities comprise large acreages of brush and woods that provide excellent tick habitat. Anthropophilic tick species *I. scapularis* and *Dermacentor variabilis* are abundant. The Soldiers typically deploy for 2-weeks training sessions and rarely leave post at each installation; they are dressed in identical uniform while training. We compared the abundance of *I. scapularis* adults vs. nymphs removed from Soldiers in the month of June. From 1997 to 2010, for example, there were 55% adults and 45% nymphs (n = 428) from Camp Ripley; 19% adults and 81% nymphs (n = 220) from Ft. McCoy; and 7% adults and 93% nymphs (n = 147) from Ft. Indiantown Gap. We also compared the infection prevalences of *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi*, and "EML." From 1997 to 2010, greater prevalences of infections of all pathogens were reported from Camp Ripley. Infection with "EML" was reported only from Camp Ripley and Ft. McCoy. Prevalence of pathogen coinfection was also greater at Camp Ripley. These three locations vary in annual temperature cycles, and seasonal synchronicity of *I. scapularis* life stages – in this case, adults and nymphs – may be driven by the differences in the amplitude of the annual cycle of maximum temperature (differences in annual temperature cycles – more extreme vs. milder seasonal climates). This seasonal synchronicity of life stages also affects pathogen infection prevalence (Gatewood et al., 2009).

A003 (EC005)

Blacklegged tick development and survivorship in the eastern United States

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In the eastern United States, Lyme borreliosis cases are caused by blacklegged ticks (*Ixodes scapularis*) that are infected with the bacterium *Borrelia burgdorferi*. Lyme borreliosis incidence is about two orders of magnitude lower in the South than the Northeast and upper Midwest. Unpublished results from multi-state surveys indicated that, although blacklegged tick populations are established in southern states, overall population densities are lower compared with the northern states. This difference in Lyme borreliosis risk may be partly due to regional differences in blacklegged tick abundance and activity via their effects on tick-host encounter rates and pathogen transmission and maintenance. We investigated the geographic variation in developmental timing and survivorship of blacklegged ticks in Wisconsin, Rhode Island, Tennessee, and Florida. In order to infer the relative roles of local adaptation and environment, we also examined how development timing and survivorship changed when ticks were transplanted among regions. Pairs of blacklegged tick adults were collected from field sites and fed on laboratory rabbits in the fall of 2011 and spring of 2012. Engorged females produced in the fall were returned to their site of origin in the following winter. Engorged females produced in the spring were redistributed via a reciprocal transplant design across sites in Wisconsin, Rhode Island, Florida, and Tennessee. Engorged females were housed individually in plastic tubes within field enclosures that allowed for airflow, precipitation, and exposure to ambient light. Enclosures were monitored every 2–3 weeks to determine oviposition timing, egg mass survivorship, hatch timing, and larval survivorship. The development timing and survivorship of spring-fed engorged females and subsequent egg masses converged within each site, regardless of female origin. In among-site comparisons, the developmental transitions occurred more rapidly at the southern sites. In the northern sites, the developmental timing of oviposition, egg hatch, and larval questing activity appeared to converge for fall and spring engorged females, although fall-fed female survivorship was lower. However, in Florida, the fall-fed females developed earlier than the spring-fed females and survivorship of the two groups was comparable. Due to a lack of available questing adults, this particular comparison could not be made for the Tennessee site. These results suggest that regional differences in abiotic factors play a key role in observed regional variation in development and survivorship of this species. Improved understanding of the underlying abiotic factors driving blacklegged tick activity and survivorship will contribute to improved regional models for predicting future Lyme borreliosis risk.

A004 (EC006)

Investigating the importance of deer for Lyme disease ecology: a natural experiment presented by lake Michigan Islands

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Lyme disease is emerging across North America as the blacklegged tick, *Ixodes scapularis* invades new areas. How the tick spreads and becomes established is of great interest for public health. White-tailed deer are believed to be the most important hosts for adult *I. scapularis* and critical for its spread and maintenance, but few opportunities exist to investigate tick and pathogen dynamics in their absence. Two Lake Michigan Islands, one with deer and one without, presented just this opportunity. These two Islands are 5 km apart from one other and ~19 km from the mainland. We compared the abundance of ticks on North and South Manitou Islands (Sleeping Bear National Lakeshore) with three mainland sites. We hypothesized that in the absence of deer, ticks, and the Lyme disease pathogen *Borrelia burgdorferi* would be detected at low levels due to dispersal by migratory birds, whereas no larvae should be detected. In 2011, we surveyed for ticks and pathogen by trapping rodents from June to September, and we surveyed for questing ticks from June to October. Sites were visited approximately once per month. We set rodent traps along 6250 m^2 transects for a total of 300 trap nights per visit. We also dragged for questing ticks along these transects, sampling 3000 m^2 per visit. We preserved all ticks collected off of hosts and the vegetation in 70% ethanol. We also collected one 2 mm ear punch per mouse per visit, which was also stored in 70% ethanol. In the laboratory, all ticks were identified to species and were assayed for infection with *B. burgdorferi* by targeting the 16S gene using quantitative PCR. Blacklegged ticks were found at all sites and relative abundance of rodents was relatively higher on Islands compared to mainland sites. Questing adult tick relative densities were the highest on the Island with deer. Although tick density was the lowest on the deer-less Island (~75 times lower than on the island with deer) and *B. burgdorferi* was not detected, contrary to our prediction, we found larvae attached to hosts, suggesting the possibility of a self-sustaining tick population in the absence of deer. Continued sampling in 2012 confirmed this finding. This intriguing result suggests the need to examine the roles of alternative mammal hosts (coyotes, rabbits) as well as of birds for maintaining Lyme disease ticks and risk on these Islands.

A005 (EC007)

Apparent range expansion of *Ixodes scapularis* in Virginia – inferences from Lyme disease case data and vector phylogenetic analysis

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Lyme disease, caused by the bacterium *Borrelia burgdorferi* and transmitted in the eastern United States by the blacklegged tick (*Ixodes scapularis*) is increasing in incidence and expanding geographically. Recent environmental modeling efforts based on extensive field collections of host-seeking *I. scapularis* predicted a coastal distribution of ticks in mid-Atlantic states as well as an elevational limit of 510 m. However, human Lyme disease cases are increasing most dramatically at higher elevation locations in Virginia, a state that is experiencing rapid emergence of this disease. Our goal was to explore the apparent incongruity between human case data and predicted and observed *I. scapularis* distribution. We hypothesized that density of *B. burgdorferi*-infected ticks should be highest in locations associated with high human disease incidence if epidemiological data represent endemic cases. However, if ticks are limited by factors associated with higher elevations, we would expect higher densities of infected ticks in locations with fewer Lyme disease cases, suggesting that cases at high elevations are either misdiagnosed or represent allochthonous exposure. To test this hypothesis, we sampled *I. scapularis* nymphs at four field sites along an elevational gradient in central Virginia. Each site was visited four times to capture temporal variation in nymphal host-seeking activity. We used PCR amplification of the ospC gene to determine *B. burgdorferi* infection status in individual ticks. We found significantly higher densities of infected ticks at our highest elevation site and overall low densities at lower elevation sites. Phylogenetic analysis of tick mtDNA gene sequences suggests that the ticks at our western and central Virginia sampling sites are more closely related to northern populations than to southern-variant *I. scapularis*, potentially indicating recent population expansion from the North rather than a re-emergence of southern populations. Our results suggest that: (1) the range of *I. scapularis* is increasing to the south, mirroring that of observed Lyme disease cases in humans, (2) *I. scapularis* may not be limited by elevation, at least along the southern Lyme disease expansion front, and (3) Lyme disease cases at higher elevation sites likely represent local exposure and enzootic maintenance of *B. burgdorferi* at these sites. Specific mechanisms to account for *I. scapuaris* range expansion have not been identified but may include dispersal and movement of the mammalian and avian host species. Ultimately, identifying the environmental drivers of tick occurrence and expansion will be critical to understanding changes in human risk to tick-borne diseases.

A006 (EC008)

Revive, a surveillance program for tick and tick-borne diseases: molecular detection of *B. burgdorferi* and *Rickettsia* spp.

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To assess the risk posed by ticks and tick-borne pathogens to Public Health is essential to have reliable data on tick population, namely main species, status, distribution, and the changing trends in tick distribution and abundance. Since 2011, the Ministry of Health requested to the National Institute of Health to implement and coordinate a nationwide surveillance program. During 2012, ticks were collected all over the year from hosts and by flagging the vegetation. Through the engagement of several health agencies, more than >10,000 ticks from 848 records across mainland Portugal have been submitted, representing 13 tick species *Dermacentor marginatus; Dermacentor reticulatus*; *Haemaphysalis punctata*; *Hyalomma lusitanicum*; *Hyalomma marginatum*; *Ixodes canisuga*; *Ixodes hexagonus*; *Ixodes ricinus*; *Ixodes ventalloi*; *Rhipicephalus annulatus*; *Rhipicephalus bursa*; *Rhipicephalus pusillus*; *Rhipicephalus sanguineus*. The majority of ticks sent to the laboratory were *R. sanguineus* (69%), followed by *R. pusillus* (16.4%) and *H. marginatum* (9.7%). The other tick species were collected in occasionally, such as *I. ricinus* with only 0.3%. Sixty-five ticks were collected on humans and *R. sanguineus* was the most frequent (41%) followed by *I. ricinus* (20%). All the human ticks and about 5% of the ticks collected from vegetation or in hosts were analyzed for the presence of *Rickettsia* spp. and *Borrelia* spp. *B. lusitaniae* and seven rickettsial species were detected, namely *R. aeschlimannii*, *R. conorii Malish*, *R. helvetica*, *R. massilae*, *R. raoulti*, and *R. slovaca*. Some ticks species were found to be only infected with a bacterial agent, as for example *H. marginatum* with *R. aeschlimannii*, and *D. reticulatus* with *R. slovaca*, when others such as *R. sanguineus* and *I. ricinus* were infected with three different agents (*R. conorii Malish*, *R. massilae* and *B. lusitaniae*, and *R. helvetica*, *R. monacensis*, *B. lusitaniae*, respectively). In the future, other tick-borne pathogens will be included in the program. Due to the high density of *Hyalomma* spp. present in Portugal and the recent detection of Crimeia Congo Haemorrhagic Fever (CCHF) in Spain, the surveillance of this virus will be implemented during this year. REVIVE program has already produced valuable results and will be maintained for at least the next 4 years that will allow to observe the trends in the Portuguese tick fauna and pathogens. Another important output of this network is to raise awareness about tick-borne diseases among the populations and the healthcare providers as medical doctors and nurses.

A007 (EC010)

Coinfection and population dynamics of *Borrelia bissettii* and *Borrelia burgdorferi* in a tick-murine borreliosis model

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Lyme Borrelia is the most common vector borne disease in the United States and is caused by spirochetes from the *Borrelia burgdorferi sensu lato* (s.l.) complex. Seven species of Lyme Borrelia have been described in the United States, yet *B. burgdorferi sensu stricto* (s.s.) is widely considered the main cause of human Lyme borreliosis. The most common vector for Lyme spirochetes is hard tick from the Ixodidea family. Several species of ticks in the United States are competent vectors of Lyme Borrelia; however, the most common vectors for human Lyme are *Ixodes scapularis* and *Ixodes pacificus*. Many other ticks circulate Borrelia in cryptic enzootic cycles. *Borrelia bissettii* is a Lyme Borrelia spirochete that is endemic to multiple regions in the US and Europe. Past studies show that certain strains of *B. bissettii* cause pathology in murine hosts, while more recently publications implicate *B. bissettii* inhuman disease. Because *B. bissettii* circulates between ticks and small rodents, in a similar fashion to *B. burgdorferi s.s.*, we assessed the ability of *I. scapularis* to transmit *B. bissettii* in a murine transmission model under both single and dual infection scenarios. Utilizing culture and quantitative PCR, we monitored the population of borreliae in the tick from larval acquisition to flat adult and measured infection loads in various murine tissues. Additionally, by monitoring growth, this study demonstrates competition between *B. burgdorferi s.s*. and *B. bissettii* in traditional spirochete media. We show that *I. scapularis* can acquire *B. bissettii* and will maintain the infection trans-stadially at numbers significantly less than *B. burgdorferi s.s.*, yet it is an incompetent vector in a murine-model. Spirochete loads in some murine tissues were significantly higher under single infection when compared to dual infection. Under dual infection, we also characterize the heterogeneous Borrelia population numbers in multiple murine tissues and describe differential acquisition of Lyme Borrelia in ticks. Description of this atypical Borrelia infection in ticks may help elucidate the biological processes undergone by the closely related human pathogenic *B. burgdorferi s.s*. in its tick vector, and expounds our understanding on the ecology of *B. bissettii* a potential emerging human pathogen.

A008 (EC011)

Vector ecology of Lyme disease in New York State: a phylogeographic approach

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In New York State (excluding New York City), the number of diagnosed Lyme disease cases has steadily increased over the past three decades, corresponding with expansion of the geographic range of *Ixodes scapularis*. The major goal of our group is to investigate the complex interactions between *Borrelia burgdorferi*, *I. scapularis*, and their natural environment that have potentially contributed to the increased geographic range of Lyme disease in New York. Twenty-seven locations in eight counties were surveyed for *I. scapularis* to determine tick abundance, and prevalence and distribution of tick-borne pathogens, over a 4-years period. A total of 11,204 *I. scapularis* (3300 nymphs, 7904 adults) were individually screened for *B. burgdorferi* using polymerase chain reaction, and the frequency and geographic distribution of *B. burgdorferi* genotypes were analyzed. Pathogen prevalence varied geographically and temporally during the time period examined, and was related to measurements of tick density. We used statistical models to evaluate the temporal and spatial heterogeneity of *I. scapularis* populations and to assess the biotic and abiotic factors that influenced species distribution and population growth, utilizing empirical data documenting growth of the tick population. Tick population density increased annually across the geographic range and was positively correlated with mild temperatures, low precipitation, low forest cover, and high urbanization. We also used population genetic tools to infer tick demographic history and migration patterns. We determined that both geographic range and tick population densities are increasing, primarily through progressive local migration events from southern populations to adjacent northern locations. Heterogeneities were found in the distribution and frequency of *B. burgdorferi* major ospC groups, with ospC group K (associated with disseminated Lyme disease in humans) occurring most frequently. Our studies can lead to a mechanistic understanding of how the environmental factors in an ecosystem determine the rate and direction of dispersal of a pathogen and its vector, influencing the corresponding geographic range of human Lyme disease. Our long-term goal is to elucidate the mechanisms contributing to human Lyme disease risk that could be targeted by ecological control strategies or educational campaigns.

A009 (EC012)

Borrelia species DNA in questing *Amblyomma maculatum* (gulf coast ticks) from Mississippi

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In the United States, *Ixodes scapularis* and *Ixodes pacificus* are primary vectors for the Lyme disease agent, *Borrelia burgdorferi sensu stricto*. Within the United States, the majority of Lyme disease cases are reported from the Northeast and upper Midwest. Few cases are reported elsewhere in the country, including the South, though the region falls within the geographic range of *I. scapularis*. In fact, in the South, the true extent of Lyme disease is under some dispute. The occurrence of Lyme disease in this region is also complicated by the existence of a Lyme disease-like illness, "southern tick-associated rash illness" (STARI). STARI is linked to the bite of *Amblyomma americanum*, the most common tick species in the South, but the agent of STARI, if any, has not been identified. *A. americanum* may harbor *Borrelia lonestari*, an apparently non-pathogenic Borrelia, but is not a competent vector for *B. burgdorferi sensu stricto*. The recent detection of *B. burgdorferi* and *B. lonestari* DNA in *Amblyomma maculatum* removed from deer and canids in Arkansas led to our current investigation of Borrelia in questing *A. maculatum*. In this study, DNA extracts of 306 unfed adult *A. maculatum*, collected from vegetation in Mississippi during 2009 and 2010, were used as template for nested PCR amplification of the *flaB* gene using Borrelia-wide primers. Amplicons were bidirectionally sequenced, and sequences were compared to those in the NCBI database. Of 306 tested tick extracts, two produced amplicons with closest identity (89 and 95%) to *Borrelia turcica*, a species first described nearly a decade ago from Hyalomma ticks in Turkey. Two additional tick extracts produced amplicons with sequences that had 100% identity to *Borrelia hermsii*, and a fifth sequence had 100% identity to *B. burgdorferi*. As *B. hermsii* and *B. burgdorferi* DNA has been used as a positive control in our laboratory, these may represent contaminants. In order to confirm these results and acquire additional information about the unknown Borrelia species identified in the two tick extracts, we will amplify a portion of the 16S rRNA gene using Borrelia genus-wide primers. However, additional experiments will be necessary to evaluate the role of the unknown Borrelia species in human disease. While the infection rate of *A. maculatum* with this uncharacterized Borrelia was quite low (<1%), adult *A. maculatum* can bite humans, possibly adding another layer of complexity to diagnosis of Lyme disease in the South.

A010 (EC013)

Frequency of rickettsiales infections in ticks collected in different regions of México

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Tick-borne rickettsial diseases (TBRD) are emerging zoonotic diseases caused by obligate intracellular bacteria and transmitted by *Ixodidae* ticks. This group is integrated by *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, *Ehrlichia canis*, and *Rickettsia rickettsii*. The distribution of the vector has been described in parts of Asia, Africa, Europe, and America. Six rickettsiae species are pathogens to humans. The transmission of these pathogens to human has been increased due to recreational activities during the Spring and Summer seasons, and the introduction of residential houses in forest areas. In Mexico, a case of ehrlichiosis and anaplasmosis, and epidemic outbreaks of Rickettsiosis have been reported, but the prevalence of TBRD and of infected vectors is unknown.

Objective: To determine the frequency of *A. phagocytophilum*, *E. chaffeensis*, *E. canis*, and *R. rickettsii* in ticks of Mexico.

Materials and Methods: DNA was extracted from 323 ticks collected from 1997 to 2012 in Northwest, Northeast, Central, and Southwest states of Mexico. Infections were determined in extracted DNA by PCR amplification with 16S genes to each agents and CS 78–323 to *R. rickettsii*.

Results: Eighty-nine (27.5%) ticks were positive for rickettsial infections, 44 (13.6%) for *A. phagocytophilum*, 40 (12.4%) for *E. canis*, 4 (1.2%) for *E. chaffeensis*, and 4 (1.2%) for *R. rickettsii*. The infected ticks were identified as *Rhipicephalus sanguineus* in 74 (83%), *Ixodes scapularis* in 4 (4.5%), *Dermacentor* spp. in 4 (4.5%), *Amblyomma cajennense* in 3 (3.3%), *Boophylus microplus* in 2 (2.2%), *Haemaphisalis leporis-palustris* in 1 (1.1%), and *A. dissimile* in 1 (1.1%). Coinfection with *A. phagocytophilum* and *E. canis* was identified in 3 (3.3%) ticks. *A. phagocytophilum* was more frequent in the Center and East of Mexico, *E. canis* in the Northwest, and *E. chaffeensis* and *R. rickettsii* in the Center of the country.

Conclusion: We report for the first time *A. phagocytophilum* and *E. chaffeensis* infection in ticks from Mexico. *R. sanguineus* was the tick most frequently infected with *Rickettsia*, although its role as a competent vector for *A. phagocytophilum* and *E. chaffensis* is unknown. Clinicians must consider rickettsial infections in febrile and hemorrhagic cases from zones endemic for infected ticks.

A011 (EC014)

Recent population growth and range expansion of blacklegged ticks in New York State

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Blacklegged tick (*Ixodes scapularis*) populations have recently been discovered in areas of the Northeastern United States once thought to be devoid of this species. The establishment of these populations may have resulted from either in situ growth of existing populations that maintained very low densities or by migration and subsequent colonization. We used Bayesian phylogeographic and demographic approaches to infer the origin and demographic history of recently detected blacklegged tick populations in the Northeastern United States. The data and results indicate that the newly detected tick populations are not the product of in situ population growth from an existing low-density population, but from recent colonization resulting in a geographic range expansion. This expansion in the geographic range proceeded primarily through progressive local migration events from southern populations to adjacent northern locations, although long-distance migration events were detected. Demographic phylogeographic inferences that both the geographic range and the population densities are increasing. The results of these analyses address critical questions concerning this medically important disease vector, including the history of the populations, the role of local and long-distance dispersal in their establishment, and the connectedness among new and old populations. These results may be critical to the development of monitoring and disease control policies in the near future.

A012 (EC015)

Prevalence and distribution of deer tick virus in cervids, Maine, USA

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Powassan virus (POW) and deer tick virus (DTV) are closely related tick-borne flaviviruses that circulate in North America. The number of POWV and DTV human infection reported to the CDC has increased in recent years suggesting increased circulation of these viruses. In 2010, we examined sera from harvested white-tailed deer *Odocoileus virginianus* and moose *Alces americana* and screened for DTV antibodies. Our aim was to find out if deer and moose are useful sentinels for DTV activity especially since they are frequently parasitized by *ixodid* ticks, the vectors for POWV and DTV. Sera from deer were collected in Maine from 13 counties statewide while moose sera were collected from three northern counties. The sera were screened for neutralizing antibody using a novel recombinant DTV-WNV chimeric virus. In white-tailed deer, 59/328 (18%) contained antibodies to DTV in 12 counties surveyed. In moose, 12/146 (8.2%) samples from three counties showed DTV antibodies. When examined at the county level, no association between antibody-positive deer and the current distribution of *Ixodes scapularis*, the vector of DTV, was noted. Overall, DTV antibody-positive sera were more prevalent in southern counties with established *I. scapularis* populations versus northern counties where the distribution is emergent [25.7% (19/74) in south vs. 15.7% (40/254) in north, chi-square test, $P = 0.05$]. Our results suggest that transmission of DTV is occurring across a wide geographic area in northern New England, despite a lack of recognized human cases in the region.

A013 (EC016)

Climate change by 2060 and range expansion of deer ticks (*Ixodes scapularis*) in Maine

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Over the past two decades, Lyme and other tick-borne diseases have increased with the range expansion of the deer tick (*Ixodes scapularis*) in Maine. However, there has been insufficient accumulation of degree days (DD) in northern counties and at higher elevations to permit larval hatch prior to immobilizing temperatures. In this study, we examined the likelihood that by 2060 *I. scapularis* will be able to complete its life cycle statewide and expand its range given climate warming. A time series of adult and nymphal deer tick submissions from the public to the Maine Medical Center Vector-borne Disease Laboratory (1989–2010), a time series of larval burdens on resident songbirds (1989–2010), and weather data from Portland allowed us to examine correlations between deer tick abundance and weather. On a monthly basis, adult *I. scapularis* were positively correlated with early (November–December) and late (March) winter temperatures and negatively correlated with snowfall and snow depth, and July nymphs were positively correlated with rainfall and negatively correlated with maximum temperature in July. August larvae were negatively correlated with Julian day at which 1241 DD >6°C were accumulated. The University of Maine Climate Change Institute used the Weather Research and Forecasting model to simulate climate over the northeastern U.S. at 24 km resolution for 2060, configured to use lateral boundary input from the global circulation model used in the Intergovernmental Panel on Climate Change Fifth Assessment Report. The models indicated that the 2060 annual temperature climates of northern, central, and southern Maine will be like present central Maine, southern Maine, and Massachusetts, respectively, and that annual rainfall in Maine will increase. We predict that by 2060, given suitable habitat and hosts: (a) milder winters (warmer with less snow) will increase the length of the questing season for adult *I. scapularis* particularly in northern and central Maine, likely resulting in increased questing and fecundity, (b) greater July rainfall is likely to prevent mortality through dessication; and (c) sufficient accumulation of DD >6°C across the state will permit timely eclosion statewide, so the life cycle of *I. scapularis* can be completed statewide. Increased incidence in Lyme disease is expected due to increases in *I. scapularis* abundance particularly in the northern six counties if warming and increased precipitation is accompanied by: (a) changes in forest cover types, (b) increases in suburbanization, and (c) peridomestic overabundance of deer.

A014 (EC017)

Stability of tularemia natural foci on Martha's Vineyard

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Type A tularemia has been endemic to Martha's Vineyard, MA, USA, for the last 12 years, with a median of five cases reported each year. More than half of the cases comprise primary pneumonic exposure, but the fomites remain unidentified. American dog ticks appear to stably maintain the agent, with 2–4% of host seeking adult ticks containing evidence of infection each year in some foci that we study. Host blood meal analyses indicate that the meadow vole feeds most subadult dog ticks, but these hosts are extremely scarce in our study sites, with typical densities of fewer than 5/ha. Because the agent of tularemia has been consistently present over the last decade, the relative absence of rodent hosts appears to be compensated for by diverse mechanisms such as transovarial transmission, co-feeding, enhanced longevity of dog ticks, as well as ancillary vectors such as deerflies. Tularemia ecology on Martha's Vineyard directly contrasts with that of the sympatric deer tick transmitted infection, particularly Lyme disease, with respect to the contribution of chronically infected rodent hosts to the stability of transmission.

A015 (EC019)

Dominance of *Borrelia lusitaniae* in mountain ecosystem in Northern Slovakia (Central Europe)

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Lyme borreliosis (LB) is the most prevalent tick-borne disease in Europe and USA. This disease is caused by spirochetes of the complex *Borrelia burgdorferi sensu lato* (s.l.) and is transmitted to humans by ticks of the genus *Ixodes*. *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia valaisiana* are the most common European genospecies. On the contrary, *Borrelia lusitaniae* predominates in countries of the Mediterranean, such as Portugal, Morocco, and Tunisia. In Slovakia, its prevalence is low and restricted to only a few sites. The aim of our research was to study the expansion of ticks into higher altitudes in mountain ecosystem and its infection with the *B. burgdorferi s.l.* genospecies. Questing ticks were collected by flagging in three altitudes: 600, 800, and 1000 m above sea level (a. s. l.) during 2004, 2006–2011. Genomic DNA of ticks was isolated by alkaline hydrolysis method. Presence of borreliae was tested by specific PCR and further genotyped by RFLP and/or sequencing. Tick abundance was highest at 600 m a. s. l, and lowest at 1000 m a. s. l. Total infection prevalence of *B. burgdorferi* s.l. was 29%. The infection prevalence of borreliae decreased from 38.5 to 4.4% from 600 to 1000 m a. s. l. *B. lusitaniae* was the most frequent genospecies (>60% of positive ticks) during all studied years (2004, 2006–2007, and 2009–2011), with the exception in 2008 when *B. afzelii* predominated (62%). In this year, *B. lusitaniae* was detected in 24.1% of positive ticks. Our study confirmed the spread of *I. ricinus* ticks into the higher altitudes – into new habitats represented by mixed and coniferous forests. Noteworthy, our study site in mountains represented natural focus of *B. lusitaniae*, which is usually associated with lizards and dry xerothermic habitats of Iberian Peninsula. Why is occurrence of *B. lusitaniae* long term and dominant in our study site? Climatic conditions, composition of vegetation and hosts are fundamentally different in comparison with dry habitats of Mediterranean. Further studies are currently undertaken to unravel the ecological associations of this endemic focus of *B. lusitaniae*.

A016 (EC020)

Emergence of *Borrelia burgdorferi*-infected *Ixodes scapularis* and *Peromyscus leucopus* in Ohio

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Currently, Ohio is considered by the Centers for Disease Control and Prevention (CDC) to be non-endemic for Lyme disease, the most common vector-borne disease in the United States. However, according to a passive surveillance program at Ohio Department of Health, the number of *Ixodes scapularis*, the tick that transmits Lyme disease, has increased sharply since 2009. All the three active stages (larva, nymph, and adult) of *I. scapularis* were found in a central Ohio area surveyed in this study. Furthermore, seven nymphal and eight adult *I. scapularis* tested positive for genomic DNA of *Borrelia burgdorferi*, the causative agent of Lyme disease. Antibodies to *B. burgdorferi* antigens were detected in 2 of 10 (20%) captured *Peromyscus leucopus* as well as 41 of 355 (11.5%) dogs residing in Ohio. Collectively, these data indicate that the enzootic life cycle of *B. burgdorferi* is beginning to establish in Ohio.

A017 (EC021)

Analysis of *Borrelia burgdorferi sensu stricto* strains from the Southeastern United States: relatedness to strains from endemic and non-endemic North American and European localities

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The first expanded analysis of *ospC* alleles of *Borrelia burgdorferi sensu stricto* from the southeastern United States demonstrated that *ospC* genotypes commonly associated with human Lyme disease in endemic European and North American regions were detected in *B. burgdorferi* strains isolated from non-human biting tick *Ixodes affinis* and rodent hosts in southeastern United States. We found that 30% of southeastern United States isolates were *ospC* allele L strains, a type previously considered to be exclusively European. Majority of the samples were cultured from *I. affinis*, ticks that usually do not bite humans and from ear clips or bladders of two major reservoir hosts of Borrelia in southeastern United States, *Peromyscus gossypinus* and *Sigmodon hispidus*. *OspC* allele L shared the frequency of distribution with *ospC* allele B strains (30.2% each). Two other ospC alleles detected among southeastern strains were alleles G and H (28.3 and 11.3%, respectively). It was believed that *ospC* allele L very rarely, if ever, causes human disease. Analyzed in this study, *B. burgdorferi ospC* allele L strains showed the ability to disseminate in two of the most common natural reservoir hosts in the south, the cotton mouse (*P. gossypinus*) and cotton rat (*S. hispidus*). The limited distribution of both primary reservoir hosts of *B. burgdorferi s.s. ospC* type L strains suggests that globally rare *ospC* allele L might be limited largely to the southeastern United States. Detection of the invasive ospC type B in 30% of samples in strains from the southeastern United States raises the question whether LD risk to humans in this region has been overlooked, or if the geographic distribution of the LD spirochete has evolved over time. The previous analysis of non-human biting *I. affinis* from southeastern United States revealed that they are heavily infected with *B. burgdorferi* (33–35%). Most probably, maintenance vectors, such as *I. affinis* could have significant impact in LD dynamics, helping to maintain high levels of *B. burgdorferi* in reservoir hosts that are later fed upon by bridge vectors often biting human. Following analysis of *B. burgdorferi* strains is necessary to clarify the molecular epidemiology of Borrelia, to evaluate spirochete-vector-host associations, and possible disease risk to humans in a geographic region where the presence of LD is controversial.

A018 (EC022)

Ecology of *Borrelia burgdorferi sensu lato* in diverse habitats of Slovakia

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In the Central Europe, *Borrelia burgdorferi s.l.* is represented by diverse spectrum of genospecies. Since different clinical manifestation and specific host associations has been assigned to different genospecies, it is crucial to exactly identify borrelia circulating in natural foci. We have analyzed genetic variability and ecological associations of *B. burgdorferi s.l.* indifferent habitats of Slovakia. More than 5500 questing *Ixodes ricinus* ticks were sampled from 10 diverse habitats including mountain spruce forest, lowland deciduous forest, xerothermic steppe, suburban forest, urban park, game reserve, and woodland-farmland ecotone. Moreover, at two sites (urban and rural) host feeding ticks collected from birds, lizards, and rodents were analyzed as well. About 1221 birds, 114 rodents, and 9 lizards were captured. In total, 21.6% of questing ticks were infected with *B. burgdorferi s.l.* The overall prevalence between different sites varied from 7.1 to 46%. Significant differences were observed within single site between different years. *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia valaisiana* were detected at each studied site as the most prevalent with the few exceptions. In sub-mountain area of central Slovakia, *Borrelia lusitaniae* constantly predominated. *B. burgdorferi sensu stricto* was not detected at every site, but it was commonly found in urban parks from both western and eastern Slovakia. At these sites, *Borrelia bavariensis* and *Borrelia spielmanii* were detected. Borrelia positive ticks in urban habitats harbored mostly *B. garinii* and *B. valaisiana* assigning blackbird population an essential role for circulation of borrelia in towns. The lowest prevalence of Borrelia was found at urban area with very low density of rodents where deer supplemented the blood meal for the *I. ricinus* nymphs and only 3.3% of adults were positive. The infection prevalence in ticks feeding on hosts was 25% in ticks from birds with *B. garinii* and *B. valaisiana*, 13% in ticks from rodents with *B. afzelii* and 26% in ticks from lizards with *B. lusitaniae* predominant. Further analysis of positive samples by RFLP, SSCP, and sequencing revealed considerable interspecific diversity including presence of two different genotypes of *B. lusitaniae* and *B. spielmanii*. Our results confirmed that local habitat structure and host composition have the highest impact on the occurrence of *B. burgdorferi s.l.* and its genetic variability.

A019 (EC024)

Spatial and temporal distribution of Lyme disease-infected ticks in the Texas–Mexico border region

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Lyme disease (LD) is the most prevalent arthropod-borne infection in the United States, with 33,097 cases of LD reported to the Centers for Disease Control and Prevention (CDC) in 2011. The disease is transmitted to a mammalian host by infected *Ixodes* ticks that carry *Borrelia burgdorferi*. There has been little effort to understand the current and future distribution of *Ixodes* ticks, and no study has focused on the Southern US and in particular in the US–Mexico border. We forecast the present and future distribution of infected *Ixodes scapularis* ticks in the US–Mexico border region of South of Texas and the Northeast of Mexico (Coahuila, NuevoLeon, and Tamaulipas). We correlate geographic data with climatic variables using a maximum entropy approach. Three different general circulatory models were used (CCCMA, CSIRO, and HANDLEY) and two IPCC scenarios (A2 and B2). One hundred models were developed and evaluated via cross validation, dividing the present data into 60 and 40% using the Area under the Curve (AUC) in an ROC plot. The final model and AUC are the average of the 100 models. Binary maps were created to assess stable habitat for *I. scapularis* in the future. The final models had AUC >0.80, which indicate that the models are robust. Our findings show suitable habitat now and in the future on the east and central parts of Texas and Northern Mexico. Consequently, these areas should be monitored to control *I. scapularis* tick infections and to assess the risk for humans to acquire Lyme disease in this geographical area. Further studies are currently being done to determine the reservoirs involved in the maintenance of this zoonotic pathogen in Southern US.

A020 (EC025)

Distribution of *Borrelia burgdorferi* in humans, dogs, and ticks in Southern USA

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Lyme Borreliosis (LB) is the most prevalent arthropod borne disease in the US with 33,097 cases reported to CDC in 2011. In 2009, the case definition of LB was revised, and currently, the CDC differentiates between confirmed and probable cases for this disease. Thus, since 2009, in Texas, the ratio of probable versus confirmed cases has been repetitively 2:1. This can be attributed to different causes, from lack of testing to the presence of genetically distinct Borrelia species, strains, and/or *Ixodes scapularis* biotypes particular to Southern US. To determine the distribution of LB in Texas, we collected ticks from deer hunting stations, veterinary clinics, and animal shelters in several counties across the state. In addition, we evaluated the sero-prevalence of Lyme disease-infected dogs in Texas during the year 2011–2012. The Texas Department of State Health Services provided with the cases of confirmed human Lyme disease in the State from the year 2000 to 2012 to compare the spatial distribution with the samples studied in this project. All ticks (574 samples) were identified to species and their DNA was purified individually. Each tick sample was tested by PCR utilizing primers specific to five genetic markers [intergenic region 16SrRNA-23SrRNA (IGR), flaB, p66, ospC, and ospA], in order to determine which *Borrelia burgdorferi* strains are circulating in Texas and how they are distributed across the state. Positive PCR results were confirmed by sequencing. Sequences were used in population genetic studies. In addition, serum from 750 dogs suspected to carry Borrelia, was evaluated by ELISA and Western blot to determine their LB status. We collected *I. scapularis* ticks from 11 Texan counties. Fifty percent of the collected *I. scapularis* were infected with *B. burgdorferi senso stricto.* Most of the infected *I. scapularis* ticks and canine samples were collected from East and Central Texas from November through March, which overlaps with an increase of outdoor activities (e.g., deer-hunting season). In addition, the distribution of infected humans and dogs co-localize around recreational areas where Borrelia infected ticks were found. Other tick species such as *Amblyomma americanum* and *Amblyomma cajenenses* were also found to be infected with *B. burgdorferi* (26.6 and 48%, respectively). Clearly, infected ticks are present in southern US and likely follow different phenology timing as compared to more northern sites. We are currently evaluating potential wildlife reservoir species and their role to maintain the pathogen in the environment.

A021 (EC026)

Borrelia burgdorferi infection of *Peromyscus leucopus*, a major reservoir of Lyme disease

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Background: The white-footed mouse *Peromyscus leucopus* serves as a reservoir for *Borrelia burgdorferi* and as a host for its tick vector in eastern North America. *B. burgdorferi* appears to be well adapted to its reservoir, as evidenced by little or no disability of their hosts and serial infections of *P. leucopus* by different *B. burgdorferi* strains. But because *P. leucopus* populations are genetically diverse, they may differ in their innate and adaptive immune responses to infection.

Methods: Laboratory-reared *P. leucopus* were infected with different *B. burgdorferi* strains, which were representative of the diversity of the species in the northeast US. Infection after 4–5 weeks was characterized by the following: (1) analysis of antibody responses to various antigens, including OspC, VlsE, and BBK07/12 proteins; (2) measurement of cytokine, chemokine, and acute phase reactants in the blood; and (3) quantitative assessment by PCR of spirochete burdens in different tissues.

Results: The experimental infections of the animals revealed both strain specific antibody responses and differences in responses between individual animals. Spirochetes were present in various tissues, but the overall burdens differed by *B. burgdorferi* strain and by individual mouse. In spite of disseminated infection, levels of selected cytokines and chemokines were not substantially elevated in infected animals compared to controls.

Conclusion: These findings could not only contribute in further understanding the ecology of *B. burgdorferi* in North America but also aid in the development and implementation of a field-based vaccine directed at *P. leucopus*.

A022 (EC027)

Tick-borne pathogens in *Ixodes ricinus* ticks collected from autumn migratory birds and breeding birds in Switzerland

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Birds are important in the ecology and circulation of tick-borne pathogens. They can disperse infected ticks over long distances but they can also act as reservoir hosts for tick-borne infectious agents. From 2007 to 2010, autumn migrating birds were captured at the Col de Jaman (VD), a pre-alpine pass situated at 1512 m above sea level and breeding birds were captured at two sites on the Swiss Plateau. A total of 4558 birds belonging to 71 species were caught and 1205 ticks were collected on them. Each tick was analyzed individually by reverse line blotting and real-time PCR for the presence of various tick-borne pathogens: *Borrelia* spp., *Rickettsia* spp., *Anaplasma/Ehrlichia*, and tick-borne encephalitis virus (TBEV). Altogether, 11.4% of birds (22 species) were infested and 39.8% of them (15 species) were carrying infected ticks. Bird species belonging to the genus *Turdus* were the most frequently infested with infected ticks. Eleven tick-borne pathogens were identified in bird-feeding ticks: six *Borrelia* species (*Borrelia garinii, Borrelia valaisiana, Borrelia afzelii, Borrelia bavariensis, Borrelia miyamotoi and Borrelia burgdorferi* ss), two *Rickettsia* species (*Rickettsia helvetica and Rickettsia monacensis*), *Anaplasma phagocytophilum*, TBEV, and *Candidatus Neoehrlichia* mikurensis. *Borrelia* spp. (19.5%) and *R. helvetica* (10.5%) were predominantly detected whereas *A. phagocytophilum* (2%), *R. monacensis* (0.4%), and TBEV (0.2%) were only sporadically observed. Interestingly, *C. Neoehrlichia* mikurensis was identified in a few ticks (3.3%), mainly from chaffinches. Results suggested that the common blackbird (*Tarquinio merula*) and the tree pipit (*Anthus trivialis*) might act as reservoir hosts for *A. phagocytophilum* and *R. helvetica*, respectively. Our study emphasizes the role of birds in the natural cycle of tick-borne pathogens that are of human medical and veterinary relevance in Europe.

A023 (EC028)

Contribution of tick phenology to geographical gradients in Lyme borreliosis in eastern and central North America

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Lyme Borreliosis is the most prevalent vector-borne zoonosis in North America, with major foci in the northeastern and north central United States, but with lower prevalence of infection in the southern states. We tested the hypothesis that the shorter active season in the north results in a two-year life cycle, with the older nymphal stage of *Ixodes scapularis* (which had been infected the previous year), active earlier in the season than the younger uninfected larval stage, whereas the longer active season in the southern U.S. disrupts this efficient transmission pattern. We sampled immature *I. scapularis* from 2010 to 2012 by flagging and dragging and from host animals at nine sites in the northeastern, north central, southeastern and south central U.S. Tick phenologies showed the hypothesized pattern from north to south along the east coast, with the classic northern (nymphs before larvae) pattern in Massachusetts, with larvae before nymphs during the summer in Florida (which would disrupt the transmission dynamic) but with some additional immature activity in early spring, and with intermediate patterns at mid-Atlantic sites. However, east–west patterns were also evident, with broad overlap of larval and nymphal phenologies in Wisconsin, and a northern pattern in Tennessee. Therefore, phonological patterns apparently contribute strongly to Lyme prevalence at some sites, but other factors are clearly important and likely predominate elsewhere.

A024 (EC029)

Diversity of *Borrelia burgdorferi sensu lato* in the southeastern United States: the history and enzootiology

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Lyme disease in the southeastern United States received little attention due to the presumably low abundance of *Ixodes scapularis* in this region and low prevalence of Lyme disease spirochete in major tick vector. The low prevalence of *Borrelia burgdorferi* in *I. scapularis* does not necessary mean the low prevalence or absence of the pathogen in the region, taking into consideration the existence of "cryptic" maintenance cycles of the spirochete in the absence of classic *I. scapularis*/*Peromyscus leucopus* transmission. Migration of infected vertebrate hosts may have a larger impact on the contemporary expansion of pathogen population than the movement of tick vectors in this region. By maintaining the spirochetes in foci where classic transmission cycle is absent, the migrating infected hosts are increasing the risk of contracting Lyme disease to humans. Cultivation of *B. burgdorferi* sensu lato from tick vectors and diverse host specimens collected in southeastern part of the United States represent a collection of more than 300 isolates from Texas, Missouri, Rhode Island, South Carolina, Georgia, and Florida. The regions of samples origin are the part of the Atlantic Flyway, one of the major bird migration routes that follow the Atlantic coast of North America. The collection includes 152 strains from six hard tick species, 131 from three rodent host species, 13 from eight bird species and a group of isolates with confirmed multiple spirochete species originated from different sources. Previous analysis of isolates revealed a presence of multiple *Borrelia bissettii* and *Borrelia kurtenbachii* strains, recently described *Borrelia carolinensis* and *Borrelia americana*, highly diverse B. *burgdorferi* sensu stricto strains and multiple *Borrelia andersonii* strains. Comparative analysis of *B. burgdorferi* sensu stricto strains collected in Lyme disease endemic and non-endemic European and North American regions revealed close relatedness of geographically distinct populations. We found that spirochete genotypes, commonly associated with human Lyme disease in endemic European and North American regions, were detected among *B. burgdorferi* strains isolated from non-human biting tick *Ixodes affinis* and rodent hosts in southeastern USA. We discovered that some *ospC* types, previously known only from Europe, are widely distributed in the southeastern USA, a finding that confirms the trans-oceanic migration of *Borrelia* species.

A025 (EC030)

Transmission dynamics of *Borrelia* bacteria in a bird tick community

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We examined the *Borrelia burgdorferi* sensu lato circulation in a tick community consisting of three species (*Ixodes ricinus, Ixodes frontalis, Ixodes arboricola*) with contrasting ecologies, but sharing a common host: the great tit (*Parus major*), one of the most common birds of European gardens and woodlands. While this bird is the principal host for the specialized nidicolous tick *I. arboricola*, it is one of the many hosts for the generalists *I. frontalis* and *I. ricinus.* We found evidence that all the three tick species have a potential to contribute to the maintenance of *Borrelia* infections in *P. major*. Field data show that the birds hosted *Borrelia*-infected larvae of both *I. frontalis* and *I. ricinus*, indicating the facilitation of *Borrelia* transmission by the songbird host. The low, but significant numbers of *Borrelia* in unfed *I. arboricola* ticks collected from bird nest boxes, provide the first field data showing that it is competent in maintaining *Borrelia* over long periods of time. Aside from the known avian genospecies (*Borrelia garinii* and *Borrelia valaisiana*), several less dominant genospecies were observed in all three ticks, including mammalian genospecies and a first record of *Borrelia turdi* for north-western Europe. In a laboratory experiment, we show that *P. major* selectively facilitates the transmission of different *Borrelia* genospecies. In this experiment, we imitated the natural situation during the bird's post-fledging period, in which *Borrelia*-naïve juvenile birds are repeatedly exposed to infected *I. ricinus* nymphs. Birds developed systemic infection of the avian genospecies, as suggested by the strong increase with infestation order in the number of infected ticks. On the other hand, birds showed a very low competence to facilitate the transmission of mammalian genospecies (*Borrelia afzelii, B. burgdorferi sensu stricto*, and *Borrelia spielmanii*) as shown by the decrease in infected fed ticks, although a low number of birds remained permissive for *B. afzelii*. In a final section, we present preliminary data of a laboratory experiment, investigating the vector competence of *I. frontalis* and *I. arboricola* for *B. burgdorferi* sensu lato. These data may complete the answer on the question whether *Borrelia* transmission cycles can be maintained by bird-specific ticks using *P. major* as host, and bridged by *I. ricinus* to other hosts.

A026 (EC032)

Identification of hosts by determining natural isotope signatures in unfed *Ixodes ricinus* ticks

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In a previous proof-of-concept study using laboratory animals, it was shown that ratios of the naturally occurring stable isotopes, 15N/14N and 13C/12C in host (gerbil, rabbit) blood were faithfully replicated in the tissues of ticks that had fed on them. Determination of natural isotope ratios in unfed ticks, therefore, has potential for identification of the hosts that they fed on in the previous stage. In the present study, larvae and nymphs of Ixodes were fed on a range of natural hosts, namely rodents (*Apodemus sylvaticus* and *Myodesglareolus*), ruminants (*Ovis aries* and *Bos taurus*), and birds (tit mice*, Parus major* and *Cyanistes caeruleus*). The natural isotope ratios in the resulting nymphs and adults were determined by elemental analysis-isotope ratio mass spectrometry, and the results examined for differences in host origins. It was found that the isotope signatures in ticks had the expected trophic shift from the signature of the relevant host bloods, so that it was possible to discriminate between ticks of different host origin. However, although the previous study suggested that it might be possible to age ticks as a result of changing natural isotope ratios over time, the present study suggested that this effect only applies to the first few weeks after the moult, during which time the ticks excrete most of the blood meal remnants from their previous blood meal. The results from this experiment suggest that it will be possible to detect and differentiate animal groups (e.g., rodents, ruminants, birds) in field-caught questing ticks.

A027 (EC031)

Spatial spread and demographic expansion of Lyme borreliosis spirochaetes in Eurasia

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The Lyme borreliosis (LB) group of spirochaetes currently comprises 18 named species that vary in their geographic distribution, host specificity, and ability to cause disease in humans. In Europe, the species *Borrelia afzelii* and *Borrelia garinii* are highly abundant, and are also regularly found in Asia. It has been shown that *Borrelia* species associated with birds, such as *B. garinii*, showed limited geographic structuring between European countries while, the rodent associated species, *B. afzelii*, showed extensive spatial structuring in Europe. Here, we included a total of 89 *B. afzelii* and 121 *B. garinii*, comprising strains from Europe and China, into the analyses. We used multilocus sequence analysis (MLSA) based on eight chromosomal housekeeping genes (clpA, clpX, nifS, pepX, pyrG, recG, rplB, and uvrA) to show that when the wider, inter-continental, distribution is considered, there is evidence of spatial structuring even in the bird-associated species *B. garinii*. Furthermore, our investigations into historical LB populations provided evidence for range expansions of *B. garinii* and *B. afzelii* populations in Europe in the distant past. We propose that the expansion of *B. afzelii* in Europe may be linked to rodent population expansions after the last glacial maximum.

A028 (EC033)

Borrelia DNA in king penguins in Crozet

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The hard tick *Ixodes uriae* parasitises an extensive range of seabird species in the circumpolar areas of both the northern and the southern hemispheres. *I. uriae* has also been shown to be infected with *Borrelia burgdorferi* sensu lato (Bbsl), the agent of Lyme borreliosis in the northern hemisphere. Fifty tick-infested adult king penguins (*Aptenodytes patagonicus* halli) breeding in Crozet Archipelago (South Indian Ocean) were examinated for Bbsl spirochetemia by *in vitro* DNA amplification. Bbsl DNA was detected from the blood of 2 out of the 50 king penguins tested, and further identified as *B. garinii*. These data represent the first report on direct Lyme borreliosis spirochetes detection from seabirds in the Southern hemisphere. Our results indicate a possible reservoir role of king penguins in the spirochete's natural maintenance and could contribute to improve our knowledge about the role of seabird species in the complex epizootiology of Bbsl and evolution of Lyme disease.

A029 (EC037)

The presence of ticks and mice infected with *Borrelia burgdorferi* and *Ehrlichia* spp. confirm Central Mexico as an endemic zone of Lyme disease and ehrlichiosis

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Lyme disease (LD) is the tick-borne zoonosis more frequently reported in the USA with an incidence of 22,000 cases per year, caused by *Borrelia burgdorferi*, transmitted by *Ixodes scapularis* or *Ixodes pacificus* ticks, and maintained by *Peromyscus* mouse. A seroprevalence of 1.1% has been reported in Mexico with the Northeast and Mexico City as the zones with higher prevalence how the Mexico City 6.2 and 3.4%, respectively. We recently reported the first cases of LD in Mexico, which patients were exposed to ticks in national parks near Mexico City.

Objective: The aim of this study was to explore if the enzootic cycle for *Borrelia* and *Ehrlichia* is present in national parks visited regularly by families.

Material and methods: During the period 2009–2011, we collected mice and ticks in the forest of three parks in central Mexico. To determine the presence of *Borrelia burgdorferi* and *Ehrlichia* infections, the mice were sacrificed and tissues (vessel, articulation, and ear) were sampled, and tick´s guts homogenized. One fraction was cultured in BSK II medium and another used for DNA extraction to amplify *fla* and *ospA B. burgdorferi* genes or 16S RNA and gp 36 *Ehrlichia* genes.

Results: 24 out of 202 (11.9%) *Peromycus* and *Neotomodon alstoni* mice were positive for *B. burgdorferi* and 28 (14%) for *Ehrlichia* sp. and 10 out 31 *Ixodes spinipalpis* and *Ixodes tovaris* ticks (30%) collected on 25 mice were positive. Amplified sequences had 95–100% homology with *B. burgdorferi* sensu stricto and 98% homology with *Ehrlichia canis* genes.

Conclusions: We confirmed that the enzootic cycles for Lyme disease and ehrlichiosis are present in national parks in central Mexico, and are maintained by *I. spinipalpis* or *I. tovaris* ticks and by *Peromyscus* or *Neotomodon* mice. The health system in Mexico needs to apply preventive measures to control the tick vectors.

A030 (EC038)

Contribution of tick phenology to geographical gradients in Lyme borreliosis in Eastern and Central North America

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Lyme Borreliosis is the most prevalent vector-borne zoonosis in North America, with major foci in the northeastern and north central United States, but with lower prevalence of infection in the southern states. We tested the hypothesis that the shorter active season in the north results in a two-year life cycle, with the older nymphal stage of *Ixodes scapularis* (which had been infected the previous year), active earlier in the season than the younger uninfected larval stage, whereas the longer active season in the southern U.S. disrupts this efficient transmission pattern. We sampled immature *I. scapularis* from 2010 to 2012 by flagging and dragging and from host animals at nine sites in the northeastern, north central, southeastern and south central United States. Tick phenologies showed the hypothesized pattern from north to south along the east coast, with the classic northern (nymphs before larvae) pattern in Massachusetts, but with larvae before nymphs in Florida (which would disrupt the transmission dynamic) and with intermediate patterns at mid-Atlantic sites. However, east–west patterns were also evident, with broad overlap of larval and nymphal phenologies in Wisconsin, and a northern pattern in Tennessee. Therefore, phonological patterns apparently contribute strongly to Lyme prevalence at some sites, but other factors are clearly important and likely predominate elsewhere.

A031 (EC039)

The effects of environmental moisture on seasonal tick abundance

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Lyme borreliosis is the most commonly reported vector-borne disease in north temperate regions worldwide; however, tick population trends and disease incidence is difficult to predict. While the importance of moisture availability to tick survival is accepted, its role in tick activity and abundance is not well understood. For example, laboratory studies have demonstrated tick mortality due to low environmental moisture, whereas some field studies have found little or no correlation between the effects of weather and tick abundance. By using data from over a decade of continuous statewide tick surveillance, we assess the effects of environmental moisture on tick survival at multiple scales. We document substantial inter-annual variability in nymphal tick abundance. Comparison of 14-year nymphal tick surveillance records for the state of Rhode Island and corresponding hourly relative humidity (RH) records for the same period of time, identified periods of sub-optimal conditions affecting tick survival. We identify tick-adverse humidity events (TAHEs), periods of sub-optimal moisture availability defined by microclimatic parameters identified in a laboratory setting. Knowledge of these microclimatic parameters are required to accurately characterize the effects of weather on tick numbers, and offer the possibility of increasingly accurate predictability of tick populations and incidence of human disease. Our findings provide the basis for the development of a weather-based early warning system to increase public awareness and target tick-borne disease preventive measures.

A032 (EC040)

Are blackbirds *Turdus merula* competent reservoirs for *Borrelia turdi* in Western Europe? A Xenodiagnostic experiment

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To confirm that thrushes, such as blackbirds *Turdus merula*, play a key role as reservoirs for some *Borrelia* genospecies in Europe, we performed a xenodiagnostic experiment with blackbirds captured in a mixed wood located in western Portugal where *Borrelia turdi*, an uncommon genospecies in Europe, is the most prevalent genospecies associated with birds. Four out of five birds transmitted spirochetes to xenodiagnostic ticks. Two birds transmitted *Borrelia valaisiana* to 25.7 and 10.5% of ticks, and two transmitted *B. turdi* to 6.4 and 5.4% of ticks. Our results showed that blackbirds transmit *B. valaisiana* and *B. turdi* to *I. ricinus* feeding larvae, acting as amplifying hosts for these genospecies in nature.

A034 (EC043)

Blacklegged tick feeding success on cotton mice and broadheaded skinks in an endemic site in Florida

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Although *Ixodes scapularis* (i.e., blacklegged tick) populations are established across the eastern United States, Lyme disease incidence is approximately two orders of magnitude lower in the south than in the north. This disparity may be attributed to geographical differences in host community composition, as host species differ in their ability to harbor and transmit the pathogen (i.e., reservoir competence) and relative impacts on tick feeding, survival, and molting success (i.e., host-specific grooming behaviors and blood meal quality). To investigate these effects, we conducted feeding trials at a research site in northern Florida, where prior field surveys showed that larvae and nymphs feed on both *Peromyscus gossypinus* (i.e., cotton mice) and *Plestiodon laticep*s (i.e., broadheaded skinks). We also investigated interactive effects between host species and tick genetics by feeding groups of larvae and nymphs that belonged to genetic clade SI (typically found in the southeast) and AmI (found throughout the eastern United States, and predominates in the north). In order to quantify feeding success, we estimated tick attachment duration, engorgement weights, and molting success. Ticks of known genetic clade were generated in a laboratory facility at Michigan State University, although supplemental trials included ticks of other ages and genetic backgrounds. Mice and skink hosts were captured directly from the field site. Feeding trials were conducted in a facility where ticks and hosts were exposed to ambient (i.e., outdoor) light, temperatures, and relative humidity. Hosts were housed in individual pens with false bottoms suspended over trays of water. Trays, food dishes, and refugia were checked every 24 h for engorged ticks until 48 h had elapsed whereby no ticks were recovered from any host. We discovered that larval and nymphal ticks that fed on cotton mice and belonged to the AMI clade began detaching earlier than ticks fed on broadheaded skinks and belonged to the SI clade (in most cases by greater than 24 h). Nymphs belonging to the SI clade and nymphs that fed on broadheaded skinks were significantly heavier on average than nymphs belonging to the AMI clade and fed on cotton mice, respectively. Due to large variation in larval weights, observed differences were not significant, although similar trends were observed. The molting of engorged ticks is currently being monitored and will be discussed. These results suggest that the host species and tick genetics have interactive effects on feeding success and therefore influence tick population establishment and maintenance.

A035 (EC044)

Blacklegged tick drop-off timing on cotton mice and broadheaded skinks

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The differential effects of different host species on *Ixodes scapularis* (i.e., blacklegged tick) vectors have been well established in the literature. Previous findings support host species differences in reservoir competence (i.e., their ability to harbor and transmit the pathogen to the blacklegged tick vector) as well as direct impacts on tick survival and molting success (e.g., grooming behaviors and blood meal quality). However, less is known about whether host species differ in terms of when ticks are likely to detach from the host. In nature, relatively small differences in host drop-off timing could have large impacts on a tick's probability of survival and future host-seeking probability, given the broad range of microhabitats that hosts encounter throughout a diurnal cycle. In order to determine whether differences in attachment duration and drop-off timing existed between hosts, we conducted feeding trials of larval and nymphal blacklegged ticks on two host species that differ in their activity periods. Experiments were conducted at a field site in northern Florida where there is an established population of blacklegged ticks. Hosts used in this study were wild-caught *Peromyscus gossypinus* (i.e., cotton mice) and *Plestiodon laticeps* (i.e., broadheaded skinks), both of which are parasitized by juvenile blacklegged ticks. Ticks used for this project derived from colonies reared in a laboratory facility at Michigan State University. Colonies comprised ticks collected from various locations within the range of blacklegged ticks as well as having different genetic backgrounds. Feeding trials were conducted in a facility where ticks and hosts were exposed to ambient light, temperatures, and relative humidity. Hosts were housed in individual pens with false bottoms suspended over trays of water. Trays, food dishes, and refugia were checked for engorged ticks every 6–8 h on a 24 h cycle until 48 h had elapsed whereby no ticks were recovered from any host. Our results reveal host species differences in tick detachment times. Both larvae and nymphs that fed on broadheaded skinks primarily detached between 6:00 a.m. and 2:00 p.m., whereas the majority of ticks that fed on cotton mice detached between 2:00 p.m. and 10:00 p.m. This pattern may be due in part to changes in host behaviors, physiological states, and/or abiotic conditions that occur throughout a diurnal period. This study has implications for understanding another aspect of how a host species may impact tick survivorship and future host-finding success via their effects on the distribution of ticks among microhabitats.

A036 (EC045)

Survival patterns of northern compared to southern genotypes of *Ixodes scapularis* in North America

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Survival patterns of northern and southern genotypes of *Ixodes scapularis* were studied under northern and southern environmental conditions in laboratory experiments. Larvae of both American clade 1 (descended from ticks collected in Wisconsin) and Southern clade 1 (from ticks collected in South Carolina) survived longer under northern than under southern conditions. These results suggest that southern environments are less hospitable for *I. scapularis* survival, which might be related to the predominantly northern distribution of Lyme borreliosis in North America. American clade 1 ticks survived longer than Southern clade 1 ticks under both northern and southern conditions in the lab. However, field data suggest higher survival of southern than northern clade ticks under field conditions in the south. Improved survival of southern clade ticks under southern conditions in the field might result from behavioral differences between clades, possibly related to host associations.

A037 (EP017)

Two natural reservoir hosts identified for *Borrelia miyamotoi* in Switzerland

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In Europe, at least 10 *Borrelia burgdorferi* sensu lato (sl) species have been identified in the tick *Ixodes ricinus*. Furthermore, *Borrelia miyamotoi*, a species related to the relapsing fever spirochetes, has been reported in questing *I. ricinus*. The mouse *Peromyscus leucopus* and passerine birds were identified to play a role in the maintenance of *B. miyamotoi* in USA. Nevertheless, no study was conducted to demonstrate potential reservoir hosts for *B. miyamotoi* in Europe. Here, we were interested in the role played by wild rodents in the maintenance of *B. miyamotoi* in Switzerland. In 2011 and 2012, small mammals were captured in an area where *B. miyamotoi* occurs at a prevalence of 2.4% in questing nymphs. *I. ricinus* ticks from small mammals were analyzed after the moult by PCR/RLB to identify the different *Borrelia* genospecies. In parallel, ear biopsies and blood samples were taken to confirm the presence of *Borrelia* in rodents and xenodiagnosis was used to evaluate their reservoir role. Most of the 110 captured rodents (95.5%) were infested by *I. ricinus* ticks and 8.18% were infested by *Borrelia* infected *I. ricinus* larvae. *Borrelia afzelii* (2.8%), *B. bavariensis* (1.6%), and *B. miyamotoi* (0.17%) were detected in the ticks attached to rodents. *B. miyamotoi* was observed by microscopy in one blood sample. Xenodiagnostic larvae fed on infected *Myodes glareolus* and *Apodemus flavicollis* revealed the transmission of *B. miyamotoi* with infection prevalence reaching 10–30%. This study showed that at least two rodent species are reservoir hosts for *B. miyamotoi* in Europe.

A038 (EP018)

Review of the pathogen diversity of human interest transmitted by *Ixodes ricinus* in Switzerland

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The tick *Ixodes ricinus* is mainly known to transmit the virus of tick-borne encephalitis (TBE) and the etiologic agent of Lyme borreliosis (LB), the bacteria *Borrelia burgdorferi* sensu lato (sl). However, additional pathogens belonging to the genera *Rickettsia*, *Anaplasma*, and *Babesia* have been reported in this tick species. The aim of this study was to review the current situation concerning these various pathogen genera in Switzerland including recent and older data. Screening of more than 110,000 *I. ricinus* collected in more than 400 different sites allowed to identify 15 different tick-borne pathogen species: seven different *Borrelia* species, two *Rickettsia* species (*Rickettsia helvetica* and *Rickettsia monacensis*), *Candidatus Neoehrlichia* mikurensis, *Anaplasma phagocytophilum*, and three *Babesia* species (*Babesia divergens, Babesia microti*, and *B. venatorum*) as well as TBE virus. Globally, *B. burgdorferi* sl and *Rickettsia* spp. were the most prevalent pathogens with infection prevalence in questing ticks varying from 23 to 50% for *Borrelia* spp. and from 10 to 21% for *Rickettsia* spp. *C. Neoehrlichia* mikurensis was identified in 6% of examined ticks. These pathogens were detected in *I. ricinus* ticks from all investigated sites. In contrast, other pathogens such as TBE virus, *A. phagocytophilum*, and *Babesia* spp. were localized at restricted areas with prevalence lower than 2%. LB is frequent in Switzerland with between 7000 and 12,000 annual cases (yearly incidence 131 per 100,000 inhabitants) and TBE reaches around 100 cases every year (yearly incidence 1.6 per 100,000 inhabitants). Concerning the other tick-borne pathogens, well-documented cases do not exist, except for *C. Neoehrlichia* mikurensis, since three human cases have been recently reported.

A039 (EP020)

Temporal and spatial distribution of Lyme borreliosis in Southern US

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Lyme Borreliosis (LB) is the most prevalent arthropod borne disease in the US with 33,097 cases reported to CDC in 2011. In 2009, the case definition of LB was revised and currently, the CDC differentiates between confirmed and probable cases for this disease. Thus, since 2009 in Texas the ratio of probable versus confirmed cases is repetitively 2:1. This can be attributed to different causes, from lack of testing to the presence of genetically distinct *Borrelia* species, strains, and/or *Ixodes scapularis* biotypes in southern US. To determine the distribution of LB in Texas, we collected ticks from different deer hunting stations, from veterinary clinics and animal shelters in different counties across the state. All ticks (574 samples) were identified to species and their DNA was purified individually. Each tick sample was tested by PCR utilizing primers specific to five genetic markers (intergenic region 16SrRNA–23SrRNA (IGR), flaB, p66, ospC, and ospA), in order to determine which *B. burgdorferi* strains are circulating in Texas and how they are distributed across the state. Positive PCR results were confirmed by sequencing. Sequences were used in population genetic studies. In addition, serum from 750 dogs suspected to carry *Borrelia*, was evaluated by ELISA and Western blot to determine their LB status. We collected *I. scapularis* ticks from 11 Texan counties. Fifty percent of the collected *I. scapularis* were infected with *B. burgdorferi* senso stricto. Most of the infected *I. scapularis* ticks and canine samples were collected from east and central Texas from November through March, which correlates with the deer-hunting season. Other tick species such as *Amblyomma americanum* and *Amblyomma cajenenses* were found infected with *B. burgdorferi* (26.6 and 48%, respectively). Genetic markers flaB and IGR were the most effective in detecting ticks infested with *B. burgdorferi.* Our preliminary population genetic study suggests that ticks positive for both markers were mostly distributed in eastern Texas. The distribution of *Borrelia* we found in ticks correlates with the distribution of the majority of canine samples that tested positive for *B. burgdorferi* in this study and with the cases of LB in humans reported to CDC since 2000.

A040 (EP026)

Ecology of *Ehrlichia muris* in the American midwest: vector–host–pathogen associations and genetic diversity of an "emerging" human and animal health threat

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In 2009, an *Ehrlichia muris*-like organism was for the first time associated with human disease in the United States, and in 2012 canine disease from infection was first reported. To begin to understand the ecology of the *E. muris*-like organism (hereafter *E. muris*) in the Midwestern US, we analyzed field samples collected from 2010 to 2012 at Fort McCoy army installation in west central Wisconsin, near the origin of the recently reported human infections. Samples were collected with a rigorous ecological sampling design to allow exploration of pathogen–vector–host associations and pathogen genetic diversity. We subjected 674 samples, including 199 ear biopsies from 4 small mammal species, 62 *Ixodes scapularis* adults removed from hunter-harvested deer, and 408 questing *I. scapularis*, to three separate PCR assays (citrate synthase, GroEL, and 16S rRNA) to detect and characterize *E. muris* infection. Small mammals exhibited low *E. muris* infection prevalence, with 3.4% of 79 *Peromyscus leucopus*, 1.5% of 65 *Clethrionomys gapperi,* and none of 55 shrews testing positive. The infection prevalence of questing nymphs (10.9%, $n = 190$) was double that of questing adults (5.1%, $n = 138$); suggesting that larval bloodmeal hosts may be reservoirs and associated with more infection than the nymphal bloodmeal hosts. Questing larvae ($n = 80$ pools consisting of 206 larvae) were characterized by 1.5% minimum infection prevalence indicating possible transovarial transmission. Adult ticks removed from white-tailed deer (*Odocoileus virginianus*) had a similar infection prevalence (4.8%) to the questing adults, suggesting that deer are not important reservoirs. Sequencing of the citrate synthase gene fragment revealed that 27/37 samples shared 100% homology with the *E. muris* organism found in all four positive controls, which were derived from ticks from WI at the time of the human outbreak. The remaining 10 samples contained one to four nucleotide substitutions from the common strain. In contrast, sequences for the other two genes (GroEL and 16S rRNA) were uniform across our samples and the positive controls. The ecology of *E. muris* in the American Midwest is complex, involving multiple reservoir hosts and co-infections with other pathogens in the *I. scapularis* microbial guild. Given the high abundance of *I. scapularis* in this region, the presence of *E. muris* in all three life stages, and the relatively high infection prevalence estimates in questing nymphs and adults, future studies must continue to elucidate the ecology of this "emerging" pathogen.

A041 (EP027)

Detection of *Borrelia miyamotoi* in *Ixodes* ticks and small mammals in Russia

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Background: The relapsing fever group spirochete *Borrelia miyamotoi* has been found in hard-body ticks in Europe, Russia, Japan, and USA. Different species are involved in transmission of *Borrelia miyamotoi* including *Ixodes persulcatus*, *Ixodes ricinus, Ixodes scapularis, Ixodes pacificus*, and *Ixodes dentatus*. We studied the presence of *B. miyamotoi* in hard-body ticks and small mammals in Russia.

Methods: Over a six-year period, *I. ricinus* (n = 1090 individuals) and *I. persulcatus* (n = 1958) were collected in different parts of Russia (Central Russia, North Caucasus, Volga River Region, Ural, and Siberia), as well as in East Kazakhstan. Small mammals were collected in the Republic of Udmurtia, Volga River Region. The most prevalent species was *Myodes glareolus* (n = 240 individuals), while 40 other mammals belonged to the *Apodemus uralensis, Mus musculus, Apodemus flavicollis*, and *Myodes rutilus species*. *B. miyamotoi* was amplified from ticks or tissue samples using specific real-time PCR, confirmed in most cases by amplicon sequencing. DNA and RNA of other tick-borne pathogens were detected using the commercial kit "AmpliSense™ TBEV, *B. burgdorferi* sl, *A. phagocytophilum, E. chaffeensis/E. muris*" (CRIE, Russia, Moscow).

Results: *B. miyamotoi* was detected 1.2% of *I. ricinus* ticks in central Russia and the North Caucasus. Higher infection rates of *I. persulcatus* were found in central Russia (1.9%, n = 628), Volga Region (5.7%, n = 544), Ural (2.9%, n = 481), and Siberia (3.3%, n = 305). *I. persulcatus* collected in east Kazakhstan had nearly the same *B. miyamotoi* infection rate (3.7%, n = 533). The 62% of *I. ricinus* ticks and 35% of *I. persulcatus* ticks, which were positive for *B. miyamotoi*, were also co-infected with *Borrelia* from *B. burgdorferi sl* group. The *B. miyamotoi* and *B. burgdorferi sl* infection rates of *Myodes glareolus* were 3.3 and 18.3%, respectively. Only one other species of rodents (*A. uralensis*) was found to be infected with *B. miyamotoi* (one of eight animals).

Conclusion: *B. miyamotoi* infection rates in *I. persulcatus* and *I. ricinus* ticks in Russia are relatively low in comparison to *B. burgdorferi sl*. About 40–50% patients with serologically confirmed *Ixodes* tick-borne borreliosis (ITBB) in Russia do no have an erythema migrans rash. In the Republic of Udmurtia and Yekaterinburg Region, we were able to detect *B. miyamotoi* DNA in blood samples of about half of the patients without erythema migrans (about a quarter of all ITBB patients). Because of the observed higher infection prevalence in humans than ticks, we conclude that *B. miyamotoi* is like other relapsing fever spirochetes, requires less time and/or fewer bacteria to infect humans compared to Lyme borreliosis spirochetes.

A042 (V014)

A novel relapsing fever *Borrelia* sp. and reptile-associated *Borrelia* sp. from amblyomma geoemydae, and its difference of the transstadial transmission

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We found novel relapsing fever *Borrelia* sp. (*Borrelia* sp. AGRF) and reptile-associated *Borrelia* sp. (*Borrelia* sp. tAG) from *Amblyomma geoemydae* in Japan. The *Borrelia* sp. AGRF was phylogenetically related to the hard (ixodid) bodied tick borne relapsing fever *Borrelia* spp., *Borrelia miyamotoi* and *Borrelia lonestari*. On the other hand, the *Borrelia* sp. tAG formed a monophyletic group and it was closely related to reptile-associated *Borrelia* spp. The prevalence of the *Borrelia* sp. AGRF and *Borrelia* sp. tAG were 14.2% (39/274) and 31.4% (86/274), respectively. In addition, these 14 ticks (5.1%) were co-infected with both *Borrelia* spp. The transstadial transmission of *Borrelia* sp. AGRF was occurred in the tick midgut and the salivary glands, although *Borrelia* sp. tAG was only detected in the tick midgut of *A. geoemydae*. The difference of the borrelial niche regarding the transstadial transmission might be associated with borrelial characterization. Thus, our findings may contribute to the elucidation of transstadial transmission mechanisms of *Borreliae* in ticks.

A043 (M003)

6S RNA in *Borrelia burgdorferi*

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Borrelia burgdorferi is transmitted from its tick vector to a vertebrate host, which requires regulating the program of gene expression via an alternative sigma factor cascade in which RpoS globally controls the genes required for the different phases of the enzootic cycle. In *Escherichia coli*, 6S RNA facilitates the switch to RpoS-mediated gene expression in stationary phase by binding to RpoD-RNA polymerase holoenzyme, which sequesters RpoD from recycling and competing with RpoS as well as regulating transcription at RpoD-dependent promoters. Perhaps not surprisingly, the 6S RNA in *B. burgdorferi* (Bb6S) does not accumulate at high cell density, as in *E. coli*. Bb6S binds *in vitro* to RNA polymerase holoenzyme from both *E. coli* and *Bacillus subtilis* (with RpoD and SigA, respectively), and we are currently working on overexpressing the *B. burgdorferi* RNA polymerase to assay for binding. We have mutated the Bb6S gene and are currently evaluating its role gene expression and the enzootic cycle.

A044 (M004)

Induction of Lyme disease spirochete death by increasing RpoS expression

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RpoS, one of the two alternative σ factors in *Borrelia burgdorferi*, is tightly regulated by multiple regulators and, in turn, controls expression of essential and critical virulence determinants. Here, we show increasing RpoS expression induces spirochete death. The immediate effect of this induction was to promote bacterial division and to result in a rapid increase in cell number before causing bacterial death. Although induction led to over 99% spirochetes dead, a very small number of bacteria were able to survive after prolonged induction of more than 10 days. Interestingly, this induction of cell death did not show DNA fragmentation, the hallmark of programmed cell death. The potential biological significance of induced cell death may help *B. burgdorferi* regulate its population and maintain lifecycle.

A045 (M006)

Bacterial metabolic activity as a rheostat to control expression of infection-associated genes

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The Lyme disease spirochete controls production of its proteins during each stage of the infectious cycle, as do all other pathogenic organisms. An early hypothesis proposed that *Borrelia burgdorferi* directly senses environmental temperature to determine expression levels of the OspC and Erp infection-associated proteins. We recently demonstrated that *B. burgdorferi* actually responds to the variations in its metabolic activity that occurs at different temperatures rather than temperature per se. This new hypothesis helps explain results of other recent studies on borrelial gene expression. Moreover, evaluation of other pathogens indicates that effect of bacterial metabolism on pathogenesis-associated factors is a wide-spread phenomenon. Both *Borrelia*-specific and broadly applicable results will be presented.

A046 (M007)

Regulation of the *Borrelia burgdorferi* DnaX–EbfC operon

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Borrelia burgdorferi EbfC is a chromosomally encoded nucleoid-associated protein that exerts global effects of borrelial gene expression. The ebfC gene forms an operon along with dnaX, which encodes a major component of DNA polymerase. Both EbfC and DnaX are maximally expressed during periods of rapid bacterial growth, and repressed when the bacteria do not divide. Although the majority of known bacterial species also possess a dnaX–ebfC operon, essentially nothing is known about how any organism controls production of these genes. We have identified a DNA-binding protein that specifically binds near the promoter 5′ of dnaX, and present results of studies on the role of this protein in borrelial production of DnaX and EbfC. Noting that all medically valuable antibiotics target bacterial metabolic targets, and that nearly all human pathogens contain a dnaX–ebfC operon, these studies may lead to development of novel, broad-spectrum antibacterial therapies.

A047 (M008)

Genome comparisons reveal conserved regulatory sequences on two constitutive plasmids in *Borrelia burgdorferi sensu lato*

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Gene regulation plays a critical role in *Borrelia burgdorferi* especially during its transitions between the tick and vertebrate environments, but *cis*- and *trans*-regulatory elements in its genome remain largely unknown. Upon the recent completion of the genome sequences of additional twenty *B. burgdorferi* sensu lato strains, we used an evolutionary comparative approach to identify adaptively evolving genes and potential regulatory elements in their genomes. Here, we report findings from a comprehensive analysis of ORF and intergenic spacer (IGS) sequences of two constitutive plasmids lp54 and cp26. The lp54 plasmid is evolutionary more dynamic than cp26 showing frequent gains and losses of genes. Twelve lipoprotein genes on lp54 are newly discovered to be associated with adaptive divergence among *B. burgdorferi* sensu lato species. We identified over one hundred long (10 bases or more) IGS sequence blocks that are perfectly conserved across eight *B. burgdorferi* sensu lato species. These conserved IGS blocks contain known or predicted *cis*- and *trans*-regulatory elements such as promoters, ribosomal binding sites, intrinsic transcription terminators, sigma-factor binding sites, and small RNAs. Orthologous ORF and IGS sequences were made publicly available via the *Borrelia* Ortholog Retriever website at http://borreliagenome.org/orth_get/. These newly identified conserved IGS sequence blocks are prime targets for investigations into the molecular mechanisms of gene regulations in the Lyme disease pathogens.

A048 (M009)

Role of signal recognition particle docking protein (FTsY) in pathophysiology of *Borrelia burgdorferi*

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Purpose: Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is the most common vector-borne disease in the US. Signal recognition particle (SRP) docking protein FTsY is one of the key components of the cellular machinery for targeting membrane proteins. Major targets for bacterial FtsY are integral membrane proteins and there is a dearth of information on the role of FTsY in the biogenesis of borrelial membranes.

Design Methods: We over-expressed the borrelial FTsY (31 kDa) homolog (BB0076) using the pET23a system, purified to homogeneity and generated mono-specific serum in BALB/c mice. FTsY deletion mutant was generated in an infectious strain *B. burgdorferi* strain B31 and complemented a functional copy of FTsY in *cis* using gentamicin resistance cassette. We have borrelial strains with the requisite genetic elements for analysis of the role of this protein in infection using the C3H/HeN mouse model of Lyme disease. Antisera against a variety of borrelial GTPases were generated and used in determining their contribution to borrelial physiology in the absence of FTsY.

Results: Immunoblot analysis of *B. burgdorferi* propagated under either tick (pH 7.6/23°C) or mammalian conditions (pH 6.8/37°C) revealed no significant differences in the levels of borrelial FTsY. Deletion of SRP-FTsY reveled a significant reduction in the levels of major outer surface protein C (OspC) suggesting that FTsY could play a role in the adaptation of *B. burgdorferi* to vertebrate host-specific conditions. This phenotype could be due to limitation in the trafficking of OspC to the membranes thereby altering the infectivity phenotype of *B. burgdorferi*. Levels of SRP-Ffh were elevated in the mutant compared to the control strains.

Conclusion: The reduction in the levels of OspC in the mutant demonstrates that the trafficking of OspC is either reduced or there are other transcriptional effects mediated due to the absence of FTsY resulting in reduced levels of this key vertebrate host specific protein. FTsY deficient mutant will be a critical genetic tool to decipher the SRP-mediated translocation of virulence related proteins of *B. burgdorferi*.

A049 (M013)

To SN(I)P or not to SN(I)P – that is no question!

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In Europe, Lyme borreliosis is caused by several species of the *Borrelia burgdorferi* sensu lato complex including *B. burgdorferi* sensu stricto, *Borrelia afzelii, Borrelia garinii*, and *Borrelia bavariensis*. It has been suggested that symptoms caused by the different species vary in their late manifestations, for example *B. afzelii* was mainly associated with skin manifestations while *B. garinii* and *B. bavariensis* have been associated with neuroborreliosis. However, there are cases that seem to deviate from this principle. To understand the relationship of *Borrelia* strains that induce a wide variety of symptoms such as erythema migrans, neuroborreliosis, and arthritis, we have re-sequenced the genome of more than 25 isolates of *Borrelia burgdorferi*. Patient isolates were obtained predominantly in Bavaria, Germany between 1985 and 2010. Using an Illumina MiSeq platform, we have applied "next generation sequencing" to these samples. This technique produces sequence information for the whole genome of microorganisms and offers unprecedented power to study biological relationships of bacterial pathogens. In recent years, methods based on next generation sequencing have become more reliable (in terms of sequence length and depth) and can now be applied economically to bacterial strains. Next generation sequencing produces a huge number of short sequence reads (50–250 bp in length) that can be mapped onto a reference genome permitting the identification of single nucleotide polymorphisms (SNPs) and these can be used to analyze microevolutionary processes, i.e., within bacterial populations, as well as the long-term evolutionary history of species. Sequence reads were mapped against full genome sequences available in GenBank. In addition, a novel algorithm, termed co-phylog (Yi and Lin, 2013), was explored to infer phylogenetic relationships between closely related species. For an initial comparison we concentrated on mutations in the core genome (omitting SNPs that were potentially acquired by recombination) to understand the pattern of descent within and between species. The data also permitted estimates of divergence times.

A050 (M016)

Metal ion homeostasis regulates virulence expression in *Borrelia burgdorferi*

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The ferric uptake regulator (Fur) family of DNA-binding proteins represses and/or activates gene expression based on metal-dependent signal sensing. *Borrelia burgdorferi* Fur homologue, also known as *Borrelia* oxidative stress regulator (BosR), is required for spirochetal adaption to the mammalian host, during which the expression of outer surface protein A (OspA), a tick colonization factor, is repressed, whereas the expression of OspC, a mammalian host colonization factor, is activated. However, it is unclear what metal(s) or even if metal(s) regulates BosR activity in the spirochete. Here, we show that perturbations to iron and copper homeostasis, either through genetic manipulation or by adjusting metal levels in the medium, significantly affect OspC expression in *B. burgdorferi,* with iron exerting a positive effect and copper exerting a negative effect. Furthermore, the effects of these metals at the cellular level correlated with their effects at the molecular level. While Fe-bound BosR is active, Cu-bound BosR is inactive in DNA-binding. Deficiency in BosR also results in an increase in cellular Cu level. This finding that BosR controls the homeostasis of a metal which regulates its DNA-binding activity has established in the Lyme disease spirochete a regulatory paradigm that is characteristic of the Fur family of metalloregulators.

A051 (M019)

Whole genome sequencing (WGS) of 22 *Borrelia burgdorferi* strains and variants to understand human invasiveness and enzootic proliferation

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Human Lyme disease is an emerging disease, it may also be a diverging disease. To better understand this, we determined the first sequence of type strain B31. We obtained NIH funding to increase the number of *Borrelia burgdorferi* and related strains we sequenced to 22. Due to the nature of the multiple plasmids in these isolates this was a challenging project that has been successful and is helping move the entire field forward not incrementally but large leaps. The information derived from these whole genome sequencing (WGS, determined by Sanger methods) is extremely useful for investigation into pathogenesis, safe vaccine development, and diagnostic tests for infection. Some of the "Lyme-associated agents," even though some of the component species do not cause human disease, include at least *B. burgdorferi*, *Borrelia garinii, Borrelia afzelii, Borrelia valaisiana, Borrelia lusitanie, Borrelia japonica, Borrelia andersonii, Borrelia turdae, Borrelia tanukii and Borrelia bissettii.* WGS has proven to be a fast and cost efficient way to attack many unknown questions simultaneously in a non-anecdotal way and provide a very firm foundation for the generation of new, informed, and testable hypotheses, which would be difficult or impossible to formulate by other means. As but one example, our initial whole-genome comparison off our *B. burgdorferi* genome sequences, demonstrated that in contrast to previous assumptions that genetic changes occurred only by point mutations, we found that closely related *B. burgdorferi* bacteria frequently horizontally exchange genetic information. To facilitate discovery of genetic variability associated with human virulence among the strains, these genomes were chosen, in part, to allow three levels of phylogenetic comparisons including between-species variations, within-species (*Bb* sensu stricto) polymorphisms, and the most recent evolutionary divergence between sister clonal groups. The sequence data have been deposited in Genbank, publications, and more analyses will be forthcoming in additional publications. Comparative analyses of the chromosomes and plasmids within and between species will be presented.

A052 (M020)

Genotyping Lyme borreliosis spirochetes

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Lyme borreliosis, the most common tick borne disease in the northern hemisphere, is caused by spirochetes of the *Borrelia burgdorferi* sensu lato (sl) group, a complex that currently comprises 19 genospecies. Whereas in North America a single genospecies, *B. burgdorferi* sensu stricto, is the cause of Lyme borreliosis, in Europe also *Borrelia afzelii* and *Borrelia garinii* have been identified and confirmed to cause the disease. However, other genospecies such as *Borrelia spielmanii, Borrelia bisettii*, and *Borrelia valaisiana* are also suspected to cause Lyme borreliosis. Our aim is to identify *Borrelia* genospecies of clinical isolates as well as from a tick collection representative for Austria. We dispose of a large number of field collected ticks from Austria as well as numerous DNA isolates from specimens of patients who had presumably Lyme borreliosis. The latter comprise DNA extracts of the last 8 years. The origin of these extracts comprises skin samples from suspected erythema migrans, as well as cerebral spinal fluid and synovia. Our study will allow a comprehensive overview of the *B. burgdorferi sl* genospecies distribution in certain specimens of patients and in ticks collected in Austria. Currently, we are using a nested PCR in combination with a newly developed nested real time PCR approach for detection and identification of *B. burgdorferi sl* genospecies. The clinically evident cases are being correlated with the PCR results in order to learn about the sensitivity of the methods.

A053 (M023)

DipA, a pore-forming protein in the outer membrane of Lyme disease spirochetes exhibits specificity for the permeation of dicarboxylates

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The Lyme disease causing Spirochete *Borreliae* are highly dependent on nutrients provided by their hosts. An important route for influx of nutrients is through porins that are situated in the outer membrane. In this study, a 36 kDa protein that functions as a putative dicarboxylate-specific porin is described. The protein was found in the outer membrane of Lyme disease *Borrelia*. It was purified by hydroxyapatite chromatography from *Borrelia burgdorferi* B31 and designated DipA, for dicarboxylate-specific porin A. The dipA gene was partially sequenced and corresponding genes could be identified in the genomes of *B. burgdorferi* B31, *Borrelia garinii* PBi, and *Borrelia afzelii* PKo. DipA also has a high homology to the Oms38 porin found in relapsing fever *Borrelia*. The black lipid bilayer assay was used to characterize DipA and the protein was found to have a single-channel conductance of 50 pS in 1 M KCl, to be slightly selective for anions and was not voltage-dependent. The channel could be partly blocked by different di- and tricarboxylic anions. The results imply that DipA forms a porin specific for dicarboxylates, which may play an important role for the uptake of specific nutrients in different *Borrelia* species. Ongoing studies that will be presented aim to further understand the function of this porin and will be achieved by mutational analysis. We will also generate expression constructs for DipA for structural and physiological studies of this porin.

A054 (M026)

Role of single lipoproteins in the formation of lipid rafts microdomains in *Borrelia*

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Borrelia burgdorferi, the agent of Lyme disease, contains numerous lipoproteins as well as free cholesterol and cholesterol glycolipids, in its outer membrane. Structural, biochemical, and biophysical analysis revealed that spirochetal cholesterol glycolipids exist in microdomains in cultured and *in vivo* derived spirochetes. Mass spectrometry analysis of detergent resistance membranes isolated from *B. burgdorferi* grown in three different culture conditions (4, 33, 37°C plus blood) showed that the protein content of each fraction was similar and several lipoproteins (OspA, OspB, and OspC) that could potentially play a structural role in the formation of these domains were identified. Other proteins detected in the lipid microdomain are involved in protein and glycolipid catabolic processes, such as HtrA, P83/100, and a LysM domain protein. Several mutants (ΔOspA, ΔOspB, and ΔOspC) were analyzed to measure the impact of lipoprotein in lipid raft formation by thin layer chromatography (TLC) and transmission electronic microscopy (TEM). Lipid extracts were resolved on an HPTLC silica plate with a chloroform–methanol solvent system and stained with iodine vapor while TEM data were analyzed using the Ripley's K-function. In addition, FRET measurements on living *Borrelia* were made as previously described by LaRocca et al. (2010) using 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate [TMA-DPH (donor)] and Octadecyl Rhodamine BChloride [ODRB (acceptor)]. Fluorescence was measured as a function of increasing temperature every 5°C from 15 to 40°C. These studies provide evidence of the molecular complexity and uniqueness of the membrane of *B. burgdorferi* and prove that single deletions of lipoproteins do not affect lipid raft formation.

A055 (M027)

Investigation of the mechanism of the requirement for P66 in *Borrelia burgdorferi* infection in mice

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P66 is an outer membrane protein of *Borrelia burgdorferi* that has been shown to have β3 integrin-binding and channel forming activities *in vitro*. The protein is essential for *B. burgdorferi* to cause infection in mice, but not ticks. Our current focus is on determining the mechanism underlying the defect in Δp66 strains. The Δp66 strains are able to survive in a mammalian host in dialysis membrane chambers implanted into the peritoneum of rats, but are cleared from the site of inoculation in skin within 24–48 h. Live imaging of wild-type (WT) RFP expressing plus GFP expressing Δp66 strains at the site of inoculation reveal that both are motile, and both rapidly disappear from the skin at the inoculation site. The WT bacteria reappear within 5–7 days, but the mutants never reappear. Quantitative PCR data are consistent with the live imaging analyses. We are currently investigating whether ablation of either macrophages or dendritic cells can rescue the Δp66 mutants, and whether the integrin-binding activity of P66, specifically, is required for *B. burgdorferi* to cause infection.

A056 (M029)

mRNA lifetime in *Borrelia burgdorferi*

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Borrelia burgdorferi, the causative agent of Lyme disease, requires shifts in gene expression to undergo its natural enzootic cycle between tick and vertebrate hosts. mRNA degradation is an important mechanism for gene regulation, but has never been studied in *B. burgdorferi*. We are examining the half-lives of individual mRNAs to determine whether mRNA half-lives correlate with gene function, particularly functions related to the enzootic cycle. We have monitored the mRNA levels of rpoS and flaB following transcriptional arrest in *B. burgdorferi*. Approximately 3 h after transcriptional arrest, rpoS levels were significantly decreased, but still detectable. However, flaB mRNA levels remain high for at least 19 h. qRT-PCR using identical amounts of total RNA were performed and indicate that flaB Ct values increase (suggesting a decrease in relative RNA levels) but are high compared to control reactions run without template or run in the absence of reverse transcriptase. These results suggest that flab mRNA remains a significant fraction of the total RNA and may indicate that the transcript is long-lived. Long-lived fragments of artificially regulated ospC and rpoS transcripts in inducible strains following removal of the inducer IPTG from culture media was also observed.

A057 (M033)

A new method for efficient delivery of bacterial pathogens into ticks

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Vector-borne bacterial pathogens that are maintained in natural enzootic cycles are of considerable public health interest. The portions of the pathogen life cycles in mammalian hosts have been studied extensively in some cases, but studies in arthropod vectors lag behind. In part, this is due to the difficulties in loading arthropods with sufficient and reproducible doses of bacteria to allow selections or screens for bacterial genes that are expressed in the vector environment. We developed a method to introduce reproducible numbers of *Francisella tularensis* LVS into *Dermacentor variabilis* nymphs. Nymphal ticks are placed on mice and allowed to feed. On the day before tick drop off, LVS is introduced into the mice by retroorbital injection, and the ticks are allowed to feed to repletion. Using this method, different doses of bacteria can be introduced into the mice, and therefore into the ticks. A purMCD mutant derivative of LVS that is not infectious in cell culture or murine models of infection was introduced into the ticks, and demonstrated to be present in the nymphs at drop off but not in freshly emerged adults. A complemented mutant in which the purMCD locus was expressed under the control of an exogenous promoter survived from the molt to the adult stage. This system will allow for a "promoter trap" selection for genes that are expressed in the tick stage of the *F. tularensis* life cycle, and is likely to be adaptable to other vector-borne bacterial pathogens.

A058 (M034)

Comparative transcriptome analysis of geographical distinct virulent and attenuated *Babesia bovis* reveals different gene expression profiles

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Babesia bovis is a tick-borne apicomplexan protozoan responsible for causing splenic fever globally not only in agriculturally important livestock, but also in human being, often mis-diagnosed for malaria. One unique feature of *B. bovis* is that virulent strains can cause cerebral babesiosis, a syndrome of neurovirulence clinically similar to cerebral malaria. In nature, a wide diversity of *B. bovis* virulent phenotypes exist but experimentally, attenuation can be induced *in vivo* by serial passages of virulent parental strain in a splenectomized host. The phenotypic characteristic of neurovirulence is gradually lost, and an attenuated derivative is obtained. Animals infected with the attenuated derivative are protected upon virulent parental challenge, indicating that determinants of virulence may be modulated independently of epitopes responsible for protective immunity. Mechanism that results in virulence loss is currently unknown. Using *B. bovis* as a model system, not only can one explore the virulence loss phenotype, downstream genomic manipulation is feasible. Recent genomic comparison between three virulent and attenuated *B. bovis* strain pairs indicated that there are no significant changes among the protein-coding genes shared between the pairs that could explain the divergent phenotypes although an overall attenuated strain genome reduction by all three attenuated strains was observed. These data suggest one of two phenomena: (i) differences between virulent and attenuated strain pairs lie in the non-coding regions, which influence transcriptomic variability between virulent and attenuated strains or (ii) the loss of genome content during the attenuation process is the key to attenuation. In this study, we investigated the transcriptomic profiles between two geographically unrelated *B. bovis* strain pairs and reports that though shared differential gene expressions are found, the overall transcriptomic signatures are distinct, suggesting multiple routes to attenuation.

A059 (V007)

Inactivation of the *Borrelia burgdorferi* BBA66 gene causes a tick transmission defect resulting in attenuated mouse infection

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Lyme borreliosis, or Lyme disease in humans, is caused by the tick borne pathogen, *Borrelia burgdorferi*. The spirochete exists in a natural transmission cycle colonizing *Ixodes* spp. ticks that acquire the organism after feeding on infected reservoir hosts, most commonly small mammals. Transmission of *B. burgdorferi* from infected nymphal ticks to a host during the bloodmeal feeding process is the key event for human infection. Defining the tick/pathogen/host interactions that facilitate vector transmission is of significant interest in developing strategies to interrupt the enzootic cycle of Lyme borreliosis. The impact of the *B. burgdorferi* surface localized immunogenic lipoprotein BBA66 on vector and host infection was evaluated by inactivating the encoding gene, bba66, by insertional mutagenesis, and characterizing the mutant phenotype throughout the mouse-tick-mouse natural cycle. The BBA66-deficient mutant isolate remained infectious in mice by needle inoculation of *in vitro* cultured organisms, but differences in spirochete burden and pathology in the tibiotarsal joint were observed relative to the parental wild-type (WT) strain. *Ixodes scapularis* larvae successfully acquired the BBA66-mutant following feeding on infected mice, and the organisms persisted in these ticks through the molt to nymphs. A series of tick transmission experiments ($n = 7$) demonstrated that the ability of mutant-infected nymphs to infect laboratory mice was significantly impaired compared to mice fed upon by WT-infected ticks (45 vs 85%, respectively; p value 0.0082). Trans-complementation of the mutant with an intact copy of bba66 restored the WT infectious phenotype in mice via tick transmission. Additionally, a statistical analysis of the number of BBA66 mutant-colonized ticks that fed to repletion as a correlation for mouse infectivity was assessed. Mice were more likely to become infected with the mutant isolate when more than four ticks fed to completion. These results suggest a role for BBA66 in facilitating *B. burgdorferi* dissemination and transmission from tick vector.

A060 (V008)

Analysis of the *Borrelia burgdorferi* transcriptome in fed nymphs reveals a unique expression profile

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Aim: To characterize and compare the transcriptional profiles of *Borrelia burgdorferi* (Bb) in fed nymphs, in a mammalian host-like environment (dialysis membrane chambers [DMC]) and *in vitro* at 37°C.

Methods: Unfed nymphs infected with Bb isolate B31 5A4 were fed to repletion on naive mice. Total RNA was isolated from a pool of five Bb-infected replete nymphs. After DNase treatment and depletion of ribosomal RNA, bacterial mRNA was amplified using the Ambion Message Amp II kit. Amplified BbRNA was hybridized to Bb strain B31-based whole genome microarrays and expression profiles were characterized and analyzed using GeneSpringGX V12.0 software. Using the same methodology, transcriptional profiles were examined for Bb cultivated in DMC and *in vitro* following temperature-shift (37°C). Microarray data were validated by real time RT-PCR of a selected subset of genes.

Results: We identified 704 Bb transcripts in fed nymphs, 894 transcripts in DMC, and 840 transcripts *in vitro*. Comparison of transcriptomes revealed 364 gene transcripts that were expressed under all three conditions; these transcripts, 66% of which are chromosomally encoded, presumably constitute the "core transcriptome" that is necessary for Bb survival. In addition, we identified 127 transcripts (94 chromosomal and 33 plasmid-encoded) that were expressed only in fed nymphs; expression of 242 transcripts was detected only in Bb grown in DMCs (88% plasmid-encoded). These genes presumably require either tick- or mammalian host-specific signals for expression. Gene-by-gene comparison of normalized expression data sets identified 106 and 292 transcripts that were differentially expressed ($p < 0.05$) in fed nymphs and DMCs, respectively, compared to spirochetes grown *in vitro*.

Conclusion: To our knowledge, this is the first report describing Bb global gene expression profiles from *in vivo* samples containing limited copies of pathogen. The data demonstrate that Bb has a dynamic transcriptome that likely changes throughout the enzootic cycle in response to temporal and host-specific environmental or physiological signals. This method should also be suitable for characterizing the transcriptomes in tissues of experimentally infected mammals.

A061 (V013)

The tick midgut protein Clone19 plays a vital role in the migration of *Borrelia burgdorferi* from the midgut to the salivary glands during a bloodmeal

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The causative agent of Lyme disease, *Borrelia burgdorferi*, is transmitted to humans by the bite of *Ixodes* ticks. It takes approximately 24–48 h before *B. burgdorferi* migrates from the tick's midgut to the salivary glands from where actual transmission to the host may occur. We have recently identified a protein, designated as Clone19 that interacts with *B. burgdorferi* and is located in the tick midgut. We used yeast surface display technology for this identification, where we expressed an *Ixodes scapularis* midgut RNA library, which was probed with *B. burgdorferi* membrane proteins. By studying expression profiles by RT-PCR we established that Clone19 was expressed to a higher extent in the tick midgut than in the salivary glands during the course of a blood meal. Inhibition of Clone19 – by dsRNA silencing – in *B. burgdorferi*-infected ticks that were fed on mice resulted in lower *B. burgdorferi* loads in the salivary glands and significantly lower *B. burgdorferi* numbers in the skin of these mice. These findings were partially corroborated in mice immunized with rabbit anti-Clone19 IgG. After confirming *in vitro* that recombinant Clone19 binds to viable *B. burgdorferi*, we are currently investigating the interaction between Clone19 and *B. burgdorferi*. Recent preliminary data show a difference in *B. burgdorferi* outer membrane expression in the absence of Clone19. Further characterization of Clone19 and knowledge on the binding of *B. burgdorferi* to Clone19 will provide insight into the mechanisms exploited by *B. burgdorferi* to migrate through the tick during a blood meal. This might lead to new strategies to prevent transmission.

A062 (P020)

The HtrA protease of *Borrelia burgdorferi* degrades outer membrane protein BmpD and chemotaxis phosphatase CheX

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The high temperature requirement A (HtrA) family of serine proteases is found in all cells from prokaryotes to primates and is a key player in protein quality control. In prokaryotes, HtrA proteins function in the periplasm to degrade or remodel damaged or improperly folded membrane proteins. A unifying feature of this family is the presence of a proteolytic domain (Ser-His-Asp catalytic triad) and either one or two C-terminal PDZ domains that mediate protein–protein interactions. *Escherichia coli* DegP, the prototypical HtrA protease, is a bi-functional molecule, which, in addition to degrading misfolded proteins, functions as a chaperone during a protein folding stress response. *Borrelia burgdorferi*, the spirochetal agent of Lyme disease, codes for a single HtrA homolog, HtrABb (BB0104), which displays 41% amino acid identity to DegP. Recombinant HtrABb exhibited physical and biochemical similarities to DegP, in that it formed a trimeric structure as its fundamental unit and degraded casein via its catalytic serine198 (a catalytic site mutant, HtrABbS198A, did not exhibit proteolytic activity *in vitro*). HtrABb and DegP have some important differences as well. Native HtrABb occurred in both membrane-bound and soluble forms. In addition, despite its homology to DegP, HtrABb could not complement an *E. coli* DegP deletion mutant. Lyme disease patients, as well as infected mice and rabbits, developed a robust antibody response to HtrABb, suggestive of a role in the immunopathogenesis of Lyme disease. In co-immunoprecipitation studies, a number of potential binding partners for HtrABb were identified, as well as two specific proteolytic substrates, basic membrane protein D (BmpD/BB0385) and chemotaxis signal transduction phosphatase CheX (BB0671). HtrABb may function in regulating outer membrane lipoproteins and in modulating the chemotactic response of *B. burgdorferi.*

A063 (P021)

BobA, from the Lyme disease spirochete *Borrelia burgdorferi*, is surface exposed and binds human fibronectin via multiple independent sites

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The eukaryotic protein fibronectin (Fn) is a common target for bacterial pathogens, including staphylococcal and streptococcal species important in causing common infections. The Lyme disease spirochete, *Borrelia burgdorferi*, has several known fibronectin-binding proteins, thereby suggesting importance of this host factor in a Lyme disease infection. Recently, another borrelial protein, BB0347, was identified as having homology to other known fibronectin-binding proteins. To analyze the potential for this protein in a mammalian infection, we created recombinant BB0347 and analyzed its binding to fibronectin *in vitro*. We were also able to determine the expression of this protein by *B. burgdorferi* in culture conditions via RT/QRT-PCR and BB0347-specific Western blotting. Additionally, we cloned peptides from the full-length protein into expression vectors to determine the location of any potential Fn-binding sites. The potential importance of BB0347 was also examined by (1) determining the sub-cellular location of the protein, (2) analyzing the known cell-binding domain of fibronectin for stearic hindrance upon treatment with BB0347, and (3) examining the immunogenicity of the bacterial protein in a mouse infection. BB0347 was found to bind Fn *in vitro*, and multiple fibronectin-binding sites facilitate this binding. Additionally, the borrelial protein is expressed by the bacterium in culture, and the expression is at least partially dependent on temperature. BB0347 was also seen to be located on the outer membrane of *B. burgdorferi* and did not interfere with the binding of fibronectin-CBD-specific antibodies to the host protein. Lastly, mice injected with live *B. burgdorferi* formed antibodies against BB0347, suggesting a potential for therapies that target this protein in a Lyme disease patient. Finally, due to its specific properties, we have decided to name the protein borrelial outer-membrane binding protein A (BobA).

A064 (P041)

Role of magnesium (Mg^{2+}) in the patho-physiology of *Borrelia burgdorferi*

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Lyme disease (LD) is caused by the spirochetal bacterium *Borrelia burgdorferi* (Bb) and is the most prevalent arthropod-borne disease in the US. The life cycle of Bb relies on the colonization of either a tick vector or vertebrate host. In order to adapt to these two hosts, Bb has developed a complex network of genetic and metabolic strategies to rapidly respond to signals received from its host-specific environments. One such strategy is the metal ion dependent transport and intracellular signaling that facilitates adaptive gene expression unique to these hosts. Bioinformatic analysis of the Bb genome revealed the presence of a potential magnesium transporter (bb0380) known as MgtE adjacent to a gene annotated as protein kinase C 1 inhibitor (bb0379). The presence of histidine triad motif (HIT) in BB0379 indicated that this protein could function as a nucleotide binding protein. By utilizing reverse transcription PCR, we showed that MgtE and BB0379 are co-transcribed in Bb under laboratory growth conditions (pH 7.6/320C). To determine the significance of BB0380 and BB0379 in patho-physiology of Bb, we over-expressed and purified recombinant MgtE and BB0379 in *Escherichia coli* and generated anti-sera in mice. Immunoblot analysis revealed that MgtE expression is higher under conditions mimicking the unfed-tick (pH 7.6/230 C) than fed-tick (6.8/370 C). Increasing the pH of the media was found to up-regulate levels of MgtE independent of temperature. Chelation of the growth medium resulting in reduced concentrations of divalent cations was found to increase the levels of MgtE with a concomitant decrease in the levels of SodA, BosR, and OspC. Both mgtE and bb0379 were deleted in Bb strain B31-A3 by replacement with streptomycin resistance marker under the control of a borrelial promoter. The *in vitro* and *in vivo* phenotypic analysis of this mutant will help determine the contributions of these genes to the patho-physiology of Bb.

A065 (D011)

A homologue of tick salivary lectin pathway inhibitor (TSLPI) in *Ixodes ricinus* protects *Borrelia burgdorferi* sensu lato from killing by human complement

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TSLPI is a salivary protein in *Ixodes scapularis* and has been shown to inhibit the host lectin complement pathway by preventing mannose binding lectin (MBL) binding. TSLPI plays an important role in transmission of *Borrelia burgdorferi* sensu stricto from *I. scapularis* to the murine host as well as acquisition from the host to the tick. *Ixodes ricinus* is the main vector for *B. burgdorferi* sensu lato in Europe. With RT-PCR based techniques we identified a TSLPI homologue in *I. ricinus* salivary glands with 93% homology at the DNA and 89% at the protein level. Moreover, a Western blot with SGE from *I. ricinus* female adult ticks (fed to repletion) probed with *I. scapularis* rTSLPI-rabbit antiserum demonstrated the native protein. A BLAST search and a phylogenetic tree indicate that the TSLPI proteins are related to numerous *I. scapularis* and *Ixodes pacificus* proteins including Salp14 (a known Factor Xa inhibitor). To confirm functional homology with *I. scapularis* TSLPI we produced recombinant *I. ricinus* TSLPI in a *Drosophila* expression system and showed that *I. ricinus* rTSLPI protects *B. burgdorferi* sensu stricto and *B. garinii* from killing by human complement. In addition, *I. ricinus* rTSLPI inhibited MBL-dependent complement activation over time in a dose-dependent manner. We have generated *I. ricinus* rTSLPI mutants lacking potential N-glycosylation sites to assess whether these are involved in binding to the carbohydrate recognition domain of MBL and the complement inhibitory effect. To conclude, we have identified a homologue of *I. scapularis* TSLPI in the main European vector of Lyme borreliosis, *Ixodes ricinus*, which protects *Borrelia burgdorferi* sensu lato from killing by the lectin complement pathway at the tick bite site. Our current and future studies aim to elucidate the mechanism underlying TSLPI's MBL-inhibitory function and whether vaccination against TSLPI could prevent *Borrelia* infection.

A066 (D054)

Sensitivity of different *Borrelia* genospecies to dog serum complement

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Lyme disease is a chronic multisystem infectious disease that is the most common arthropod-borne infectious disease both in Europe and in the United States. The disease is caused by a group of spirochetes collectively known as *Borrelia burgdorferi* sensu lato. This group of microorganisms is composed of three closely related, most pathogenic subspecies – *Borrelia burgorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*. While *B. burgdorferi* sensu stricto is the cause of virtually all Lyme diseases in North America, *B. garinii* and *B. afzelii* prevail in Europe. The existing problems in diagnosis and treatment of Lyme disease and inability to effectively control and reduce the distribution of *Borrelia* vectors gave rise to an urgent need to manufacture a vaccine that would be capable to effectively immunize susceptible species of domestic animals, especially dogs, against infection with *Borrelia burgdorferi* sensu lato. Protective efficiency against each genomospecies of *Borrelia* in a vaccine must be proved by a challenge test on target species prior to registration and approval by state authority. Infection of target species has to be performed via infected ticks. The European Pharmacopoeia contains no special monograph describing efficacy testing of vaccines against borreliosis for veterinary use. Totally, 22 different *Borrelia* strains were tested for their sensitivity to dog serum complement. The borrelial immobilization, bleb formation, and bacteriolysis under dark field microscope were observed, number of live *Borrelia* cells after 8 days of cultivation was calculated and color change of suspension during cultivation was measured. Complement mediated killing of *Borrelia* in OspA and OspC antibodies-free dog serum is important for selection of *Borrelia* strains for preparation of monospecifically and artificially infected ticks for the challenge test. The aims of our work were to select suitable *Borrelia* strains for use in the challenge experiments in mice and dogs. These selected strains must unambiguously invoke specific clinical and serological changes, typical for Lyme borreliosis. Accurate mastered method of the challenge test and the efficacy assessment of vaccine against Lyme borreliosis will be used to prepare a draft monograph and its submission for approval to the European Pharmacopoeia.

A068

Whole genome sequencing of *Ixodes ricinus*, the European Lyme disease vector

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Ixodes ricinus is the most common tick species and most important vector of human and animal pathogens in western Europe. In contrast to extensive studies in *Ixodes scapularis*, the most important vector of *Borrelia* in North America, information about the most important western European tick *I. ricinus* is mostly limited to epidemiology and ecology. In the last decade, genomics has become a central discipline of biomedical research and genomic approaches are also increasingly applied to study hematophagous arthropods. Data from an *I. ricinus* transcriptome shotgun sequencing of salivary glands are available on NCBI and the genome of *Ixodes scapularis* has already been sequenced in a multiannual genome project as community effort by several investigators. In this study, the genome of its European counterpart, *I. ricinus*, has been sequenced. The sequence has been generated by second generation sequencing using the Illumina Hi Seq 2000 (Illumina, San Diego, USA of Ambry Genetics, Aliso Viejo, USA). DNA was sheared and DNA fragment ends were repaired and phosphorylated using the Klenow fragment, T4 DNA polymerase, and T4 polynucleotide kinase. Then, an adenine was added to the 3′ end of the blunted fragments and ligation of illumina Adapters was performed. Gel purification was used to size select the ligated products, which were then PCR amplified using illumina paired-end primers. The library size and concentration were determined using an Agilent Bioanalyzer. Data analysis is based on more than 700,000,000 obtained sequences probably yielding an average six-fold coverage. Sequences will be aligned to references of *I. scapularis* (whole genome contigs) and *I. ricinus* (salivary gland transcriptome library). If necessary, (partial) de novo analysis will be performed. After genome annotation, all sequence data will be made available to public databases such as NCBI or VectorBase. These genome data will provide the basement for identification of *I. ricinus* genes and molecules that will facilitate understanding of physiological processes and tick–host as well as tick–pathogen interaction.

A069 (P008)

Pathogenesis of Lyme neuroborreliosis: the response of oligodendrocytes to *Borrelia burgdorferi*

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Lyme neuroborreliosis (LNB) is a form of Lyme disease that affects both the central and peripheral nervous systems, with debilitating outcomes. In a rhesus model of LNB, we have previously shown that brains of rhesus macaques that were inoculated with *Borrelia burgdorferi* exhibit inflammatory signs, as evidenced by release of inflammatory mediators, and by oligodendrocyte and neuronal cell death. *In vitro* analysis of this phenomenon indicated that while *B. burgdorferi* can induce inflammation and apoptosis of oligodendrocytes per se, microglia are required for neuronal apoptosis. We hypothesized that the inflammatory milieu elicited by the bacterium in microglia or oligodendrocytes contributes to the apoptosis of neurons and glial cells, respectively, and that downstream signaling events in NFkB and MAPK pathways play a role in these phenotypes. To test these hypotheses in oligodendrocytes, of which little is known, several key pathway inhibitors were used to determine their effect on inflammation and apoptosis, as induced by *B. burgdorferi*. Inhibition of the ERK pathway in the presence of *B. burgdorferi* had the most profound effect on reducing inflammation, followed by JNK, p38, and NFkB pathways indicating that while multiple pathways are involved in eliciting inflammation, the MEK/ERK pathway is predominantly utilized. In addition to eliciting inflammation, low doses of *B. burgdorferi* also elicited an upregulation of total p53 protein, and suppression of the ERK pathway mitigated this effect. While inhibition of p53 had a minimal effect in reducing inflammation, suppression of the ERK pathway or p53 reduced apoptosis as measured by active caspase-3 activity and TUNEL positivity. Thus it is likely that while inflammation and apoptosis in oligodendrocytes are predominantly mediated through the MEK/ERK pathway, they are also two divergent arms of this pathway.

A070 (P009)

Early type I interferon response in mouse skin correlates with *Borrelia burgdorferi* dissemination

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Borrelia burgdorferi is the extracellular spirochetal agent of Lyme disease, the most common tick-transmitted infection in the United States. Inoculation of *B. burgdorferi* into the skin can lead to the establishment of localized or disseminated infection. Previous work in this laboratory demonstrated that *Borrelia burgdorferi* induces the production of type I interferon (IFN) by human peripheral blood mononuclear cells (PBMCs) *ex vivo*. This response was found to be dependent on *B. burgdorferi* genotype and plasmid content. Based on this observation *ex vivo*, we assessed type I IFN expression during early infection *in vivo*. C3HeJ mice were intradermally needle inoculated with *B. burgdorferi* of varying genotypes. Skin snips were collected from the site of inoculation 24 h later and real-time RT-PCR was used to assess transcript levels for IFN-β and the IFN-responsive genes mx1, ifit1, ifit3, irf7, cxcl10, stat2 and gbp1. Infection with B515, an RST1 isolate that induces high levels of IFN-α in human PBMCs and disseminates in mice, led to significant upregulation in transcript levels of type I IFN-responsive genes in 24-h mouse skin snips ($p < 0.01$, all). Conversely, B331, an RST3 isolate that does not disseminate in mice or induce IFN-α in human PBMCs, and B31-4, a lab strain lacking all linear plasmids, were unable to elicit this effect. Live spirochetes were cultured from 24-h skin snips from all B515-infected mice, as well as from 3 of 4 B331-infected mice, indicating that lack of a type I IFN response in the latter group was not due to spirochete clearance. Further, none of the mice infected with B31-4 or B331 developed disseminated infection, whereas 5 of 6 B515-infected mice did ($p < 0.001$). This indicates a link between the ability of *B. burgdorferi* to induce type I IFNs and capacity to disseminate, suggesting that there are inherent differences in the interactions of different *B. burgdorferi* genotypes and the host at the site of infection.

A071 (P010)

Conditional deletion of MyD88 during either acute or chronic phases of *Borrelia burgdorferi* infection exacerbates murine Lyme borreliosis

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Toll-like receptor (TLR) recognition of *Borrelia burgdorferi* (Bb) is a key factor controlling pathogen burden in Lyme borreliosis. Previous studies have shown that Bb infection of mice deficient in TLR2 or the TLR intracellular adaptor molecule MyD88 develop up to 100-fold increases in spirochete burdens relative to wildtype (WT) mice. In this study, we generated conditional MyD88 mutant mice (C57BL6 MyD88fl/-Esr1cre/wt[B6Cre+]) that can be rendered MyD88 deficient by tamoxifen treatment to determine whether MyD88-dependent immunity influences pathogen burdens after spirochetes have disseminated and adaptive immune responses have been generated. WT B6 mice, Cre+ mutants, and Cre-littermates were inoculated intradermally with 10e4 Bb914, a transformant of Bb strain 297 constitutively expressing GFP under the control of the flab promoter. Groups of mice were injected intraperitoneally with tamoxifen administered in oil daily for 10 days or with vehicle only during the acute dissemination phase (within 3 weeks) or the persistent phase (12 weeks) of infection. Splenic macrophages from tamoxifen-treated Cre+ mice produced lower levels of TNFα upon *in vitro* stimulation with Bb lysate and mice treated early in infection had increased IgG1 and reduced IgG2a reactivity on immunoblot, consistent with reduction in MyD88 expression. The majority of Cre+ mice treated with tamoxifen during the acute or chronic phase of infection were bacteremic on the last day of treatment, whereas Bb was cultured from the blood of only one of the WT and Cre-control mice. Bacteremia associated with MyD88-deficiency was transient, however, because none of the Cre+ mice had positive blood cultures when analyzed 10 days after completion of tamoxifen treatment. Pathogen burdens in ear tissues of Cre+ mice rendered MyD88-deficient were increased, as assessed 10 days after completion of tamoxifen treatment by qPCR for Bb DNA and by visualization of higher spirochete numbers in ear skin with two-photon intravital microscopy. Tamoxifen treatment of chronically infected Cre+ mice also resulted in elevated pathogen burdens in the heart and ear tissues, without effects on Bb-specific IgG isotypes. Preliminary results show that conditional deletion of MyD88 also results in increased amounts of GFP deposits at cartilaginous sites in the ear and knee joints in comparison to WT or sham-treated Cre+ mice. These results demonstrate that MyD88-dependent immunity is not only necessary to control pathogen burdens and eliminate spirochete antigenic remnants when Bb first establishes infection, but also during a persistent phase when spirochetes have colonized tissues and Bb-specific IgG titers are high.

A072 (P011)

Tick-specific borrelial antigens appear to be up-regulated in American but not European patients with Lyme arthritis, a late manifestation of Lyme borreliosis

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Borrelia burgdorferi (Bb) sensu lato, the etziologic agent of Lyme borreliosis, adapts to distinct environments in the mammalian host and the tick vector by differential gene expression. As a result, infected mice are not exposed to and rarely make antibodies to the set of antigens that are preferentially expressed in the tick, including outer surface protein A (OspA), Borrelia iron and copper-binding protein A (BicA), and OspD. Surprisingly, however, antibodies to OspA and BicA have been noted in American patients with Lyme arthritis. Here, we examined serum samples from 210 American patients and 66 European patients with a range of early or late manifestations of Lyme borreliosis and found that only American patients with Lyme arthritis commonly had antibody responses to OspA, BicA, and OspD. This suggests that infection with American but not European *Borrelia* strains often leads to concerted up-regulation or de-repression of tick-specific spirochetal antigens in these patients.

A073 (P012)

CspA orthologs of serum-resistant Lyme disease spirochetes are multifunctional complement inhibitory molecules

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Many important human pathogens overcome the destructive effects of complement by recruitment of fluid-phase complement regulators. Particularly, *Borrelia burgdorferi*, *Borrelia afzelii*, and *Borrelia spielmanii* resist complement-mediated killing by binding of CFH and FHL-1. Recently, the CFH and FHL-1-binding CspA protein has been identified as the key molecule of *B. burgdorferi* conferring resistance to complement-mediated killing. Owing to their property to bind these regulators, it has been suggested that CspA of *B. afzelii* and *B. spielmanii* is responsible for serum resistance, but so far closer examination of the CspA proteins of *B. afzelii* and *B. spielmanii* is lacking. Here, we investigate and compare the different binding capacities of the CspA orthologs for CFH and plasminogen and further look at their ability to inhibit complement by either binding these fluid-phase regulators or independently of these regulatory molecules. A serum-sensitive *B. garinii* strain was used to generate transformed spirochetes, ectopically producing CspA orthologs to examine their function in serum resistance *in vivo*. Recombinant CspA orthologs were used to analyze the inhibitory effect on complement activation and to assess their potential to inactivate C3b by binding of complement regulator factor H (CFH) and plasminogen. Irrespective of their species origin, all three CspA orthologs impart resistance to complement-mediated killing when produced ectopically in a serum-sensitive *B. garinii* strain. All three CspA orthologs simultaneously bound CFH and plasminogen. CspA orthologs differ in regard to their capacity to inactivate C3b via bound plasmin(ogen) and inhibit formation of the membrane attack complex (MAC). CspA of *B. afzelii* binds plasmin(ogen) and inhibits the MAC more efficiently than CspA of *B. burgdorferi* and *B. spielmanii*. Taken together, CspA orthologs of serum-resistant Lyme disease spirochetes act as multifunctional evasion molecules that inhibit formation of the MAC and inactivate C3b by binding CFH and plasmin(ogen).

A075 (P019)

An *ex vivo* skin model to study the role of skin dendritic cells in the early immunopathogenesis of Lyme borreliosis

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Borrelia burgdorferi sensu lato, the causative agent of Lyme borreliosis, is transmitted into the skin of the host during a tick bite. In the skin, two different types of dendritic cell (DC); epidermal Langerhans cells (LCs) and dermal dendritic cells (DDCs), are among the first cells of the immune system to come into contact with *Borrelia*. These cells patrol the skin for potential pathogens, which are recognized via pattern recognition receptors (PRR), upon which they become mature and migrate out of the skin. In draining lymph nodes, DCs prime T cells launch an adaptive immune response against the invading pathogen. Thus, DCs form a crucial link between the innate and adaptive immune system. It is of paramount importance to study DC subsets in their natural environment. Therefore, in order to study the role of skin DCs in the early immunopathogenesis of Lyme borreliosis, we set up a human *ex vivo* model using full-thickness donor skin. *B. burgdorferi* sensu stricto strain B31 was injected intradermally into the skin, mimicking the transmission of *Borrelia* via a tick bite. Subsequently, we studied the migration of LCs and DDCs out of the skin 24–72 h after inoculation, the activation profile of migrated cells by flow cytometry and measured cytokine production. We found a partially dose-dependent significant increase in the migration of both LCs and DDCs in response to injection with spirochetes over time, compared to injection with PBS. A preliminary experiment revealed that inhibition of Toll-like receptor (TLR)-2 – the TLR largely responsible for the recognition of *Borrelia* – with a blocking antibody inhibited the increase in migration. DCs migrated from skin inoculated with high numbers of spirochetes also expressed slightly, but significantly higher levels of the maturation marker CD83 and produced higher levels of multiple pro-inflammatory cytokines. No difference was observed in the expression of HLA-DR or CD86. Finally, we were able to culture *Borrelia* from inoculated skin 48 h after inoculation, demonstrating that the DCs are interacting with live spirochetes in this model. In conclusion, we have shown that inoculation of human skin with *Borrelia* enhances migration of LCs and DDCs out of the skin. The migrated cells are more mature and produce higher levels of pro-inflammatory cytokines than cells migrated from skin injected with PBS. This model is currently being applied as a new tool in further studies on the role of these cells in the immunopathogenesis of Lyme borreliosis.

A076 (P023)

Differential adhesion of Lyme disease spirochetes to human keratinocytes

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Upon entry into the human host, spirochetes have developed numerous strategies to successfully colonize host tissues and survive in an unfavorable environment. Adhesion to human cells is thought to be a key step for the establishment of an infection causing multiple clinical symptoms including serious neurological as well as long-term dermatological manifestations. Infection of the various tissues depends on the ability of spirochetes to bind to different cell types. In this study, we examined the ability of Lyme disease spirochetes belonging to five distinct human pathogenic genospecies (*Borrelia burgdorferi, Borrelia afzelii, Borrelia garinii, Borrelia spielmanii, and Borrelia bavariensis*) and two genospecies with disputed pathogenic potential (*Borrelia lusitaniae* and *Borrelia valaisiana*) to bind to human keratinocytes. Among the genospecies analyzed, *B. valaisiana* and *B. spielmanii* showed the strongest attachment while *B. bavariensis, B. garinii,* and *B. afzelii* displayed moderate binding, suggesting a role of these five genospecies in mediating infection of the human skin. By contrast, *B. burgdorferi* and *B. lusitaniae* showed no binding at all. Furthermore, intraspecies differences have been observed among *B. garinii, B. bavariensis, B. afzelii*, and in particular *B. valaisiana*. Recently, it has been shown that complement regulator-acquiring surface proteins (CRASPs) bind to components of the extracellular matrix. To further assess the role of these infection-associated borrelial outer surface proteins in mediating interactions with human cells, *B. garinii* cells producing distinct CRASPs originally derived from B. *burgdorferi* were screened for binding to keratinocytes. Interestingly, binding capacity for human keratinocytes increased up to four-fold when testing a *B. garinii* strain producing CRASP-4 but not CRASP-1, CRASP-2, or CRASP-3 compared to wild-type *B. garinii* cells lacking CRASPs. Taken together, these data provide evidence that distinct borrelial genospecies differ in their ability to attach to human keratinocytes and, in addition, support a role of certain CRASPs as potential adhesins of Lyme disease spirochetes.

A077 (P027)

A surface protease of Lyme disease bacteria degrades host ECM components and induces inflammatory cytokines

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Borrelia burgdorferi is deposited into the skin during the feeding of an infected tick. Systemic infection develops as *Borrelia* migrates through tissue extracellular matrices (ECM) to colonize various parts of the body. The paradigm in Lyme disease research has been that *B. burgdorferi* does not produce proteases capable of damaging host tissues. Consequently, borrelial migration and the inflammation-related pathologies observed in Lyme disease have been attributed to an exuberant host inflammatory response to the bacteria. Here, we identify and characterize a surface-exposed protease of *B. burgdorferi* (BbHtrA) that is expressed in human disease and conserved within the major Lyme disease spirochetes. BbHtrA degrades fibronectin and numerous proteoglycans found in skin, joints, and neural tissues. BbHtrA degradation of fibronectin releases highly pro-inflammatory fibronectin fragments FnIII13-14 and Fnf-29. These bioactive fragments may amplify the inflammatory processes triggered by the presence of the bacteria itself. When this hypothesis was tested directly by exposing chondrocytes to BbHtrA *in vitro*, inflammatory cytokines (sICAM-1 and IL-6) and chemokines (CXCL1, CCL1, CCL2, CCL5, and IL-8) that are hallmarks of Lyme disease were induced. Furthermore, these responses required the proteolytic activity of the protease. Together, these data suggest that *B. burgdorferi* has the capacity to degrade the host ECM and thereby participate directly in its own dissemination. Additionally, BbHtrA proteolytic activity may exacerbate the host inflammatory responses observed in Lyme disease.

A078 (P028)

Pyruvate protects *Borrelia burgdorferi* from hydrogen peroxide killing and enhances infectivity in a murine model of Lyme disease

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Borrelia burgdorferi, the causative agent of Lyme disease, encounters reactive oxygen species (ROS) stress during its enzootic cycle in the arthropod and mammalian host. Although *B. burgdorferi* lacks known ROS-defensive enzymes such as peroxidase or catalase, this pathogen was thought to be resistant to H_2O_2 due to the near absence of intracellular iron which prevents DNA damage due to the Fenton reaction. In this report, we demonstrate that pyruvate, a potent H_2O_2 scavenger, can promote *B. burgdorferi* resistance to H_2O_2. When extracellular pyruvate is absent, *B. burgdorferi* is sensitive to even a low dose of enzymatically generated H_2O_2 by glucose oxidase (GOX). H_2O_2 killing coincided with a disruption of the intracellular NAD+/NADH balance, suggesting that the killing is at least partially due to a redox imbalance. Furthermore, we showed that pyruvate could enhance the infectivity of *B. burgdorferi* in a murine model, through the reduction of ROS generated by human neutrophils. Our findings suggest that pyruvate, in addition to its classical role in metabolism, serves as an important H_2O_2 scavenger for organisms that lack enzymatic defense against H_2O_2. Extracellular pathogens may exploit the metabolite pyruvate, present in blood and tissues, to defend against H_2O_2 during infection.

A079 (P031)

Heterogeneity of *Borrelia burgdorferi* ss population and its involvement in *Borrelia* virulence: study on a murine model

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Borrelia is responsible for multisystemic disorder in humans, with an initial cutaneous inflammation, the erythema migrans then disseminated manifestations in the skin, the joint, and the central nervous system. Since the skin seems to be a key organ in the development of Lyme borreliosis and might define the organotropism, we compared the skin inflammation of different clinical isolates (human pathotypes) of *Borrelia burgdorferi* sensu stricto in C3H/HeN mouse. All the pathotypes showed an intense multiplication in the skin at day 7, pointing out the role of the skin as an amplifying site for *Borrelia* during the transmission. We found differences in their dissemination to the target organs in mice, more interestingly a very different inflammatory gene profile in the infected skin. In addition to host factors, *Borrelia* infecting strains might be constituted of a heterogenous population of bacteria that might influence its virulence in humans. To better investigate this hypothesis, we carried out a cloning on BSK-modified agar medium of *Borrelia burgdorferi* ss, strain 297. Among the different clones, we selected one for its high inflammatory profile in the skin and its speed of dissemination in mice. A precise proteomic analysis by electrophoresis and mass spectrometry revealed specific proteins for the virulent clone studied, not detected in the native strain. We further investigated the kinetics of the expression of these specific proteins in the mouse. Such an approach could constitute an original strategy to identify *Borrelia* proteins essential in the transmission and development of the bacteria in the vertebrate host.

A080 (P033)

Autophagy modulates *Borrelia burgdorferi*-induced production of IL-1beta

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Borrelia burgdorferi sensu lato is the causative agent of Lyme disease. Recent studies have shown that recognition of the spirochete is mediated mainly by TLR2 and NOD2, which have been associated to autophagy. Since IL-1beta is linked to the pathogenesis of Lyme disease, we examined the role of autophagy for the production of IL-1beta in response to *B. burgdorferi*. Our results demonstrate activation of autophagy in GFP-LC3 expressing HeLa cells shortly after exposure to *B. burgdorferi*. Human PBMCs treated with autophagy inhibitors showed an increased IL-1beta and IL-6 production in response to *Borrelia* exposure, while TNFalpha production was unchanged. Autophagy induction against *B. burgdorferi* was dependent on reactive oxygen species (ROS) since cells from patients with chronic granulomatous disease (CGD), which are defective in ROS production, also produced elevated IL-1beta compared to healthy controls. Further, the enhanced production of the proinflammatory cytokines was due to the elevated mRNA expression in the absence of autophagy. Since several studies indicated a link between high concentrations of IL-1beta and pathogenic Th17 cells leading to increased joint damage in rheumatoid arthritis (RA) or psoriasis patients, we hypothesize that the induction of autophagy could be beneficial during the chronic phase of Lyme disease leading to a decreased IL-1beta production and inhibition of Th17 cells. In summary, we have elucidated the link between *B. burgdorferi* recognition and an important regulatory mechanism of inflammation by autophagy in Lyme disease. These findings may reveal novel therapeutic targets to treat Lyme disease patients in the future.

A081 (P034)

Interferon-γ secreted by *Borrelia burgdorferi* exposed CD56+ NK(T) cells promotes keratinocyte-derived β-defensins and psoriasin (S100A7) production

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Borrelia burgdorferi sensu lato is the causative agent of Lyme disease. It has been shown that *Borrelia*-derived antigens can stimulate CD56+ NK(T) cells to produce several proinflammatory cytokines. Since NK(T) cells are abundantly present in the skin of humans, we investigated whether NK(T) cells derived cytokines can activate keratinocytes to produce proinflammatory mediators. This interaction between NK(T) cells and keratinocytes may be of importance in the development of the erythema migrans (EM) that occurs after infection of humans with *B. burgdorferi*. Our results demonstrated that CD3–CD56+ NK cells are the main producers of *B. burgdorferi*-induced IFN-γ production by human primary PBMCs rather than CD3+ CD56+ NK-T cells. Apart from IFN-γ, NK(T) cells produced high levels of both IL-1β and IL-6 after *in vitro* exposure to *B. burgdorferi*. It reveals that CD56+ NK(T) cells isolated from Lyme disease patients produce more IFN-γ than NK(T) cells from healthy individuals, when exposed to *B. burgdorferi*. To investigate the cross-talk between *B. burgdorferi* activated NK(T) cells and human keratinocytes, we performed *in vitro* experiments, in which keratinocytes were exposed to culture supernatants of *B. burgdorferi* stimulated NK(T) cells. After exposure to these NK(T) cell supernatants for 48 h, the mRNA expression of hBD2, hBD3, and psoriasin was determined in the keratinocytes. The mRNA expression of hBD-2 and psoriasin was strongly upregulated in keratinocytes and to a lesser extent hBD3. It has been demonstrated that psoriasin is involved in skin inflammation and therefore psoriasin might contribute to the development of EM. In addition, both hBD2 and hBD3 have been linked to chemotaxis of proinflammatory cells, such as monocytes and macrophages, and therefore may support skin inflammation. Future research is warranted to identify the precise role of NK(T) cells originated IFN-γ in the development of EM. Novel insights in the cross-talk between NK(T) cells and keratinocytes may result in a better understanding of the mechanisms behind the formation of the classical erythema migrans after infection with *B. burgdorferi*.

A083 (P045)

Understanding barriers to *Borrelia burgdorferi* dissemination during infection using massively parallel sequencing

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Borrelia burgdorferi is an invasive spirochetal pathogen that can cause acute and chronic infections in the skin, heart, joints, and central nervous system of infected mammalian hosts. Following transmission into a mammalian host through the bite of an *Ixodes scapularis* tick, the bacteria establish infection at the inoculation site and then quickly disseminate to distal tissues initiating long-term colonization. In this process, *B. burgdorferi* encounters multiple potential barriers to infection including adapting to environmental changes in nutrients, pH, and temperature, breaking through tissue barriers to invasion and dissemination, and evading host immune responses. Little is currently understood about which barriers have the largest effect on *B. burgdorferi* infection. A population bottleneck is an event in which the size of a population is temporarily, stochastically reduced. Bottlenecks during infection represent points at which the pathogen experiences a barrier to establishing and maintaining infection. To identify population bottlenecks during *B. burgdorferi* infection of a murine host, we adapted a quantitative massively parallel sequencing strategy, Tn-seq, for studies of *B. burgdorferi* virulence. This technology allowed us to quantitatively measure the frequency of individual *B. burgdorferi* strains within a mixed population of transposon mutants. Tn-seq is sensitive and reproducible. Individual mutants present at frequencies as low as 0.1% could be reliably detected within a population. This is a versatile technique that can be used to answer an array of open questions in *B. burgdorferi* biology. Both wild type mice and mice lacking the toll-like receptor adapter molecule MyD88 were infected with a pool of infectious *B. burgdorferi* transposon mutants with insertions in the same gene. At multiple time points post-infection, bacteria were isolated from the mice and the composition of the *B. burgdorferi* populations at the injection site and in distal tissues determined. We identified a population bottleneck at the site of infection that significantly altered the composition of the population. The magnitude of this bottleneck was reduced in MyD88-/- mice indicating a role for innate immunity in limiting early establishment of *B. burgdorferi* infection. There was not a significant bottleneck during the colonization of distal tissues suggesting that founder effects are limited and there is not a strict limitation on the number of organisms able to initiate populations at distal sites. The observed lack of host barriers to disseminated infection may account for the environmental and clinical observations that mammalian hosts can be infected with multiple strains of *B. burgdorferi* simultaneously.

A084 (M012)

Macrophage inflammatory response to *Borrelia burgorferi* is diminished in the presence of mouse sera compared to sera from deer and humans

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Lyme disease is maintained in the northeastern United States in enzootic cycles of *Borrelia burgdorferi* sensu stricto (Bb ss) by *Ixodes scapularis* and vertebrate reservoir hosts. Resistance to infection (ability of an infected host to limit microbial burden) and resilience to infection (ability to limit disease) may determine which hosts are effective (reservoir competent). White-footed mice (*Peromyscus leucopus*) remain Bb-infected for months whereas white-tailed deer (*Odocoileus virgnianus*) appear highly resistant to infection and do not contribute to maintenance of the enzootic cycle. One hypothesis for differences in resistance and resilience among species is that proteins in serum modulate the response of macrophages to Bb differently. We investigated whether macrophage inflammatory response to Bb was diminished in the presence of mouse sera relative to sera from deer, humans, and other species. Mouse bone marrow-derived macrophages (BMDMs) or human peripheral blood mononuclear cells (PBMCs) were stimulated with Bb or Pam3Cys in the presence of either mouse, human, deer or calf serum. Mouse serum suppressed Bb- and Pam3Cys-induced TNF more than human, calf, or deer serum. Bb also induced significantly less TNF after incubation in mouse whole blood compared to in human whole blood. These data are consistent with the hypothesis that persistent infection of mice with low accompanying morbidity could be at least in part due to a relative decrease in the response to Bb by immune cells in mouse tissues or blood compared with those of deer or humans, with consequently less secondary inflammation that would lead to clearance of the organism.

A085 (M024)

Borrelia miyamotoi infection of *Peromyscus leucopus* and *Mus musculus*

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Borrelia miyamotoi and *Borrelia lonestari* are two species in the relapsing fever (RF) cluster of the genus *Borrelia*, but they are transmitted by hard ticks instead of soft. The role of these and closely related species, like *B. theileri*, in human disease was doubted until recent reports from Russia and the United States provided evidence of *B. miyamotoi* infection in humans as well as their reservoir species. *B. miyamotoi* infection is of particular public health concern, because its reservoirs and tick vectors, such as *Ixodes scapularis*, are the same as those for Lyme disease species and other pathogens in North America, Europe, and Asia. However, little is known about the biology and pathogenesis of this species in either rodents, or the mammals, or humans. We previously found that the genetic diversity of *B. miyamotoi* appears to be more limited than for Lyme disease species, but this was based on only a few loci. Further characterization of the genome of *B. miyamotoi* and the identification of markers for genotyping are underway. We also previously reported that in natural infections of the white-footed mouse *Peromyscus leucopus, B. miyamotoi* and *B. burgdorferi* occupy different niches, with *B. miyamotoi* behaving like other RF species in reaching higher densities in the blood and *B. burgdorferi* reaching higher densities in the skin. Further studies of *B. miyamotoi* during experimental and natural infection are in progress. These include investigation of innate and adaptive immune responses in *P. leucopus* and the laboratory mouse *Mus musculus* and further characterization of the courses and extents of infection. The results may provide for further understanding of the ecology of *B. miyamotoi* and the pathogenesis and immunity of infection in reservoir hosts and in humans.

A086 (M032)

A mathematical model of the population dynamics of *Borrelia burgdorferi* in Lyme disease

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Although studied extensively in human patients and animal models, the intra-host dynamics of the tick-borne bacteria of the genus *Borrelia* are not fully understood. *Borrelia* exhibits a remarkably high infectivity, despite being killed efficiently *in vitro* by human phagocytic cells. In experimentally infected mice, the first immune response almost clears the infection at the injected skin site. However, the bacterial population recovers ~1 week post infection and reaches an even larger concentration before it stabilizes at lower levels. We developed a mathematical model describing the bacterial growth to explain the bacterial dynamics, including a simple phenomenological model of innate and adaptive immune processes. According to our model, the peculiar dynamics at the infection site as measured in published experimental studies cannot be explained by bacterial migration processes or a limitation in the capacity of phagocytic cells alone. The mathematical model predicts that *Borrelia* recovers from the strong initial immune response by adapting to the host environment and that this adaptation process is critical to the pathogen's survival inside its host at the very first stage of the disease.

A087 (V005)

Factor XA-induced activation of Factor V is of paramount importance in initiating the coagulation system: lessons from a tick salivary protein

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Background: Generation of active procoagulant cofactor FVa to the enzyme FXa to form prothrombinase complex is a pivotal initial event in blood coagulation and has been the subject of investigative effort, speculation, and controversy. The current paradigm assumes that FV activation is initiated by limited proteolysis by traces of (meizo) thrombin.

Methods and Results: Recombinant tick salivary protein TIX-5 was produced and anticoagulant properties were studied using plasma, whole blood, and purified systems. We here report that anticoagulant tick salivary protein TIX-5 specifically inhibits FXa-mediated FV activation involving the B-domain of FV and show that FXa activation of FV is pivotal for plasma and blood clotting. In line, tick feeding is impaired on TIX-5 immune rabbits displaying the *in vivo* importance of TIX-5.

Conclusions: Our data elucidate a unique molecular mechanism by which ticks inhibit the host's coagulation system. Based on our data, we propose a revised blood coagulation scheme wherein direct FXa-mediated FV activation occurs in the initiation phase during which thrombin-mediated FV activation is restrained by fibrinogen and inhibitors.

A088 (V009)

The role(s) of RNA sensing TLRs and adapter molecules in the host defense to *Borrelia burgdorferi* and in *B. burgdorferi* RNA-mediated type I IFN induction

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It is well established that both TLR2 and MyD88 assume critical roles in host defense to *Borrelia burgdorferi*. TLR2-/- mice contain hundreds additional *B. burgdorferi*, whereas MyD88-/- mice harbor thousands more spirochetes within their ankle joints when compared with the bacterial burden present within wild-type mouse ankle joints. These data suggest that other TLR(s) may participate in the host defense to *B. burgdorferi*. We recently discovered that *B. burgdorferi* RNA is a potent immunostimulatory ligand for mouse bone marrow-derived macrophages (BMDMs). Based on this finding, we examined whether TLR7, a MyD88-dependent receptor for single-stranded RNA, was required for clearance of *B. burgdorferi* from mouse ankle joints. Equivalent numbers of *B. burgdorferi* were detected by quantitative real-time PCR within the ankle joints of wildtype and TLR7-/-C57BL/6 (B6) mice sacrificed at 4 weeks post-infection. *B. burgdorferi*-specific serum IgG levels at 4 weeks post-infection also did not differ between wildtype and TLR7-/- animals. Type I IFN-stimulated gene transcript (ISG) levels were also assessed within B6 and TLR7-/- mouse joints at 1-week post-*B. burgdorferi* infection. In accordance with a previous report, B6 ankle joint cells did not induce the Type I ISG Oasl2. However, Oasl2 transcription was markedly upregulated within TLR7-/- ankle joints. Type I ISG transcript levels were induced to similar levels in wildtype B6 and TLR7-/- BMDMs following stimulation with either live *B. burgdorferi* or *B. burgdorferi* RNA. Taken together, our data indicate that TLR7 is not required for *Borrelia burgdorferi* clearance from mouse ankle joints or for Type I IFN-responsive gene induction. Current studies are examining the role(s) of the TLR adapter molecules MyD88 and TRIF in the Bb RNA-mediated BMDM Type I IFN response.

B001 (V002)

Neural correlates of depression in PTLDS

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Depressive symptoms, common among patients with post-treatment Lyme disease syndrome (PTLDS), have been described by some investigators as subjective-likely to have little relationship to Lyme disease. Neurobiological data from a treatment study of post-treatment Lyme disease syndrome (with encephalopathy) (ePTLDS) allow a further examination of depressive symptoms in terms of neural activity and Bb-specific antibody reactivity in serum and the intrathecal space.

Methods: The sample, drawn from an NINDS-funded study of ePTLDS, consists of 25 patients (age 45 ± 13 years and 56% female). Beck Depression Inventory (BDI) self-report assessed depression. Bb-specific antibody measure was the C6 ELISA (serum, CSF and intrathecal antibody Index – Immunetics C6-AI). The measure of neural activity is regional glucose metabolism (rCMRglu-PET). Functional regional interactions associated with depression are modeled using Principal Component Maps of covarying regional metabolism (PC-maps). Changes in nodal interactions associated with differences in intrathecal antibody reactivity are modeled as ANOVA interactions between PC-maps and C6-AI. To conserve statistical power, ANOVA is restricted to the three PC-maps that account for the largest patient variance in rCMRglu-PET.

Results: C6-AI was elevated in 15 patients and non-elevated in 10. Group mean BDI scores are comparable, as are each group's range of scores from mild to severe. $p < 0.005$ for the ANOVA prediction of BDI based on the PC Maps and C6-AI results ($R^2 = 0.68$). PC-Maps 2 and 3 have significant main effects ($p < 0.005$ and 0.01, respectively) and significant interactions with C6-AI ($p < 0.05$ and 0.005, respectively). Patient activity in PC-Map 2 is correlated with depression severity in patients with non-elevated C6-AI ($r = 0.80$); but not in patients with elevated C6-AI ($r = 0.24$). The reverse is true of PC-Map 3: $r = 0.04$ in patients with non-elevated C6-AI; and $r = 0.66$, for elevated C6-AI. PC-Map 2 reveals strong functional interactions between amygdala, nucleus accumbens, midbrain areas (PAG and VTA), brain stem (NTS), hypothalamus, hippocampus, thalamus, and anterior cingulate Cg25. PC-Map 3 reveals a different functional pattern of interactions, mainly between amygdala, PAG and thalamus. (PC-Map correlations with neuropsychological tests and secondary self-reports are non-significant.)

Conclusion: The findings indicate that depressive symptoms reported by ePTLDS patients have objective neural correlates and suggest that the Bb-specific immune status plays an important role in mediating the brain activation pattern for depression. Moreover, the identified correlates of ePTLDS depression represent prominent features of the animal and human neural networks of major depressive disorder. As new results, the next step is replication.

B002 (V010)

Clinical and immunological characteristics in relation to different *Borrelia burgdorferi* s.l. species in patients with Lyme neuroborreliosis

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Background: In Europe, Lyme neuroborreliosis (LNB) is most often caused by *Borrelia garinii*, but other *Borrelia* species have also been isolated from cerebrospinal fluid (CSF) of LNB patients. Time to recovery varies despite antibiotic treatment, possibly due to variations in pathogenicity between *Borrelia* species, as well as divergences in the host inflammatory response. The immune response within CNS in LNB is mainly of the T helper (Th)1 type with involvement of B cells, supported for example by high levels of the B cell-associated chemokine CXCL13. Furthermore, there are indications of an intrathecal Th17 response in some LNB patients, as mirrored by elevated CSF-IL-17 levels. The aims of this study were: (1) to assess the intrathecal and systemic adaptive immune response in LNB patients by measuring several Th1-, Th2-, Th17-, and B cell-related chemokines and cytokines; (2) to identify clinical and laboratory parameters related to clinical outcome; (3) to try to establish the causative *Borrelia* species and relate the findings to clinical and immunological data.

Methods: Two hundred two patients investigated for suspected LNB during 2007–2009 were included and stratified into four groups: (1) 59 with positive *Borrelia*-specific antibody index (AI) and CSF pleocytosis (confirmed LNB); (2) 21 with positive *Borrelia*-specific AI without CSF pleocytosis (possible late LNB); (3) 21 with negative *Borrelia*-specific AI but with CSF pleocytosis and clinical symptoms strongly suggestive of LNB (possible early LNB); (4) 101 without *Borrelia*-specific AI or pleocytosis (non-LNB controls). Clinical data were collected from medical charts. Serum and CSF will be analyzed during spring 2013 for CXCL8, CXCL10, CXCL13, CCL20, CCL22, IL-17, BAFF, and APRIL by Luminex technology and ELISA. AI will be assessed for several *Borrelia* species-specific antigens using the recomBead *Borrelia* kit for Luminex. CSF will be analyzed for *Borrelia*-specific nucleic acid using PCR.

Preliminary Results: Dominating symptoms in group 1 were headache, facial palsy, fatigue, and radicular pain. Group 2 had generally more unspecific symptoms of longer duration, e.g., fatigue, headache, and paresthesia. Group 3 consisted mainly of children presenting with recent onset of facial palsy and/or meningitis. Most of the patients in groups 1–3 recovered within 1–3 months, altogether 10% had symptoms lasting more than 6 months despite treatment.

Conclusion: Post-treatment symptoms lasting over 6 months were associated with higher age, longer-lasting symptoms prior to treatment and headache, tiredness, vertigo and paresthesia. Further analyses may reveal whether immunological parameters or *Borrelia* species are associated with clinical outcome.

B003 (D002)

Identifying barriers to chemical-based tickborne disease prevention measures: a survey of health fair participants in Fairfield County, Connecticut

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Background: Chemical measures, including the use of repellents, rodent-targeted bait boxes, and residential acaricidal sprays, are among the myriad recommended prevention measures aimed at preventing tickborne diseases. Despite published data suggesting such measures are effective at reducing tick populations and/or preventing human tick bites, the use of chemical measures is not widespread. A survey was conducted to help identify potential barriers to the use of such chemically based tickborne disease prevention measures.

Methods: Participants at health fairs throughout Fairfield County, CT, were asked to complete an anonymous survey regarding the effectiveness, safety, and cost of using chemical measures for tickborne disease prevention. Respondents were queried about their beliefs pertaining to the use of DEET vs. "natural" repellents, permethrin-treated clothing, residential acaricide sprays, and rodent-targeted bait boxes.

Results: A total of 216 attendees at 10 health fairs completed the survey. Of these, 88% were concerned about a family member acquiring a tickborne disease and 77% practiced some form of tickborne disease prevention. Most respondents were aware that people use DEET-based repellents (84%), residential acaricides (79%), and botanical "natural" repellents (71%) as a means of preventing tickbites. In contrast, fewer participants were aware that permethrin clothing spray and rodent-targeted bait boxes could be used for Lyme disease prevention (41 and 29%, respectively). Less than half of the respondents agreed that any of the chemical measures were safe to be used around adults, children, and pets. Less than half of the respondents agreed that the measures were safe for the environment. With the exception of DEET-based repellent use, the majority of respondents were unsure if these measures were effective for preventing tick encounters.

Discussion: This survey assessed beliefs of health-seeking individuals from a highly disease-endemic region. Most respondents were concerned about risk for tickborne diseases and were aware that repellents and residential yard sprays were used for prevention. Most respondents, however, also questioned the safety and efficacy of these measures. The majority of respondents were not aware of use, efficacy, or safety of permethrin clothing sprays and rodent bait boxes. The data suggest the need for increased education and research regarding chemical measures for tickborne disease prevention.

B004 (D007)

Nanopolymer encapsulation of a vaccinia virus-vectored reservoir-targeted vaccine for Lyme disease enhances safety and delivery

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The incidence of Lyme disease is steadily increasing in the United States and in Europe. The development of strategies to control this infection remains an important challenge. In this context, vaccination of reservoir hosts is an approach that may help decrease carriage of *Borrelia burgdorferi* and thus reduce incidence of human disease. We have previously reported protective efficacy of a vaccinia-virus expressing OspA vaccine. This vaccine, administered orally to *Peromyscus* mice as a single dose, confers protection against both transmission of *B. burgdorferi* to mice as well as protection against acquisition of spirochetes by larval ticks feeding on infected mice. Although other virally vectored reservoir targeted vaccines have been released into the environment with little environmental impact, distribution of replication competent viruses does pose an exposure risk for humans and non-target animals. In addition, much of the viral dosage administered with the vaccine is killed by gastric acid in the stomach. To overcome these issues, we are developing vaccines encapsulated with pH nanopolymers designed to degrade at specific locations in the digestive tract. We have now shown that encapsulated virus can be successfully inactivated by the nanopolymer coating and can released in its infectious form after incubation in fluid in the correct pH. Animal studies are on-going and we anticipate that we will have data to present at the conference on the efficacy of different nanopolymer encapsulated vaccines by the time of the conference.

B005 (D008)

Tick bite prophylaxis: a pilot survey to understand health care provider practices

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Background: In a recent CDC survey of over 2000 primary care providers, >30% reported prescribing tick bite prophylaxis in the prior year. To better understand current practices, we surveyed health care providers (HCPs) to determine how frequently and for what reasons they prescribed tick bite prophylaxis. Additionally, we sought to determine how often health care providers prescribed prophylaxis when they felt it was not indicated.

Methods: We included four questions regarding tick bite prophylaxis in the DocStyles 2012 survey, a computer-administered questionnaire of 3149 U.S. HCPs (primary care physicians, pediatricians, and nurse practitioners). We combined responses with information on provider demographics and practice type. Responses of HCPs in 14 Lyme disease highly-endemic states, defined as those in which incidence exceeding the 2011 national average of 7.8 cases/100,000 population, were compared with those of HCPs in other states.

Results: Of the 1485 respondents, 56% reported prescribing tick bite prophylaxis at least once in the previous year, including 74% of HCPs in highly-endemic states and 48% in other states. The most common reasons for prescribing prophylaxis in both highly endemic and non-highly endemic areas were “to prevent Lyme disease” (87 and 69%, respectively); “patients request it” (37 and 43%, respectively); and “to prevent other tickborne diseases” (21 and 35%, respectively). Of HCPs who provided prophylaxis in the previous year, 45% did so even when they felt it was not indicated. Given a hypothetical scenario involving a patient with an attached tick, 38% of HCPs from highly endemic states and 15% from other states would prescribe a single dose of doxycycline as prophylaxis; 19% from highly endemic states and 27% from other states would prescribe a full course of doxycycline.

Conclusion: A large proportion of HCPs prescribe tick bite prophylaxis for diseases other than Lyme disease, and in areas where Lyme disease is rare. Despite their specificity, tick bite prophylaxis guidelines may be difficult for providers to implement. HCPs may not be prepared to identify an *Ixodes scapularis* tick at a specific life stage; identify the degree of engorgement in the absence of tick attachment estimates; or estimate the local rate of *I. scapularis* infection with *Borrelia burgdorferi*. Additionally, patients may not want to adopt a “wait and see” approach, particularly if they lack health insurance or have a high co-pay. More information is needed regarding the efficacy of tick bite prophylaxis for diseases other than Lyme disease.

B006 (D009)

An immunoinformatics-based "genes to vaccines" approach for accelerated anti-tick vaccine discovery

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Tickborne diseases (TBDs) are a world-wide concern and constitute serious public health problems in vital need of solutions. Ticks can cause significant disease in humans and domestic animals as they carry more than 20 emerging or Category A–C pathogens. Additionally, many species of ticks are infected with and are capable of transmitting multiple agents, even in a single bite. Rather than target each individual pathogen transmitted by ticks, development of a broad-spectrum vaccine against the tick itself could prevent tickborne pathogen transmission, and such an anti-tick vaccine approach could represent a major milestone for improving public health. It is well established that ticks and other arthropod vectors manipulate host immune responses by secreting proteins from their salivary glands during blood feeding, while previously tick-bitten hosts, including humans, "fightback" by expressing an acquired immunity to these proteins. Experimental and epidemiological evidence shows that such naturally-acquired resistance to tick feeding (example, acquired tick resistance) through various cellular immune mechanisms can effectively inhibit tick blood feeding and significantly diminish or even prevent pathogen transmission and infection. Using a genes-to-vaccine platform to accelerate vaccine candidate discovery, we present data on immunoinformatic-derived peptides mined from the *Ixodes scapularis* salivary gland transcriptome as anti-tick vaccine candidates. Peptide epitopes were selected for their predicted ability to stimulate cell-mediated immunity, ability to enhance robust antibody-mediated immunity in humans, as well as for their demonstrated HLA binding capacity. Immunization in a novel HLA transgenic mouse model and subsequent tickborne pathogen challenge is providing *in vivo* validation of candidate peptide immunogenicity, and is identifying potential immune correlates of protection against *Borrelia burgdorferi* transmission.

B007 (D015)

N-terminally disulfide-bridged OspC protects mice from infection with *Borrelia burgdorferi* B31 better than its monomeric homologue

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Introduction: OspC, a plasmid-encoded outer membrane protein with high inter-strain diversity, has been studied as a candidate antigen for the development of a functional Lyme vaccine by several groups. In prior studies, the use of recombinant OspC has generally led to measurable protection against *Borrelia* expressing the autologous sequence in mouse models. However, most approaches failed to produce long-lasting protection or cross-protection against strains expressing other OspC variants, and in some studies no protection was observed. To overcome these limitations, chimeric fusion proteins of different OspC variants have been constructed, but immunoprotection studies have not been reported. The analysis of sera from OspC-immunized mice showed IgG reactivity against a major non-conserved peptide epitope in the central exposed part of OspC ("loop 5") that was not associated with protection in animals.

Materials and Methods: In this study, N-terminally (Cys19) disulfide-bridged dimeric OspC (with a Cys131Ser point mutation and an N-terminal His tag) and a comparable monomeric OspC (Cys19Gly) based on the sequence of *Borrelia burgdorferi* B31 were expressed in *Escherichia coli*. The proteins were used to immunize C3H/HeN mice in combination with alum; four immunizations at 2-week intervals were used in most experiments. At the end of the immunization schedule, the mice were needle-inoculated with *B. burgdorferi* B31, clone 5A4. Two weeks postinoculation, mice were sacrificed and tissues were analyzed for viable bacteria by culture experiments. Different quantities of OspC were tested. Serum samples were collected at different time points and analyzed by Western blot using a B31 membrane extract and ELISA using recombinant OspC.

Results: Both the dimeric and monomeric forms of OspC produced strong pan-IgG responses against the immunizing protein. After four immunizations with the dimer (0.1–10 µg per injection), nearly all animals (28/30, 93%) were protected from infection without any adverse effects. In contrast, a low proportion of mice immunized with monomeric OspC in this same dose range were protected against infection (3/30, 10%). Nodules appeared at the injection site when ³10 µg of the recombinant proteins were injected. Immunized animals developed mainly IgG1 against OspC, whereas the anti-OspC response in infected animals was characterized by high IgG3 levels.

Discussion: N-terminally disulfide-bridged OspC was much more effective than the monomeric OspC homologue in inducing a protective immune response against needle-inoculated *B. burgdorferi* B31. Additional investigations are needed to determine immunization efficacy in tick inoculation and cross-strain protection, and to uncover the mechanism of this increased protective activity.

B008 (D017)

Use of topical antimicrobial agents for preventing dissemination of *Borrelia burgdorferi* (BB) in a mouse-infection model of Lyme disease

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Background: Based on recently published reports including our own, discrepancies exist on whether topical application of antibiotics at the site of tick attachment would be a useful option for preventing cutaneous and extracutaneous Lyme disease (LD). We hypothesize that by modifying the murine animal model for LD, using a hairless but immunocompetent mouse strain (in an attempt to optimize antimicrobial penetration through the skin), it will possibly be better to determine minimally effective treatment regimens, given topically or by other parenteral routes, for preventing Bb infection.

Methods: Separate groups of 6–12 weeks old SKH (hairless) mice were infected intradermally (i.d.) in the abdominal area with 100,000 culture-grown Bb, of human isolate strain BL206. One hour and 24 h later, the following agents were applied topically over the site of inoculation: (i) a 10% povidone/1% iodine (PVI) solution; (ii) an erythromycin ointment; and (iii) a tetracycline gel formulation. Another group of similarly infected mice received two intramuscular or i.d. injections (given 18–24 h apart) of either saline or ceftriaxone (CTX: 50 mg/kg/dose), starting 1 day after borrelial inoculation. Two to 4 weeks after treatment, cultures of the urinary bladder and skin tissue from the ear were established in BSK medium in order to determine the presence or absence of Bb in both treated and untreated control mice.

Results: It was found that topical applications of PVI, erythromycin, and tetracycline were unable to prevent Bb dissemination to the urinary bladder or ear. *In vitro* studies also showed that PVI was rapidly borreliacidal at a dilution of up to 1:200 from what was applied to the inoculation site. In addition, the two-dosage regimen of CTX was 100% effective in sterilizing the urinary bladders and ear skin of the Bb-infected mice, and such treatment did not cause any renal damage based on histopathologic analyses. It is also noteworthy that, consistent with past studies, none of the mice developed any rashes at the Bb-inoculation site.

Conclusion: Our findings indicate that the topical application of various antimicrobial preparations, that are acceptable for use in humans, was unable to prevent syringe-transmitted infection in a mouse model of LD, but short-course treatment with CTX that was given by injection was curative.

B009 (D020)

Investigation of a vaccine targeting Lyme borreliosis in Europe

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In Europe, the three *Borrelia* species, *Borrelia afzelii*, *Borrelia burgdorferi*, and *Borrelia garinii* are the main species causing the most common tick-borne zoonosis, Lyme borreliosis. The heterogeneity between the *Borrelia* species and within *B. garinii* makes the search for conserved antigens providing broad protection challenging. By applying a genomic approach relying on human antibodies, we have identified 122 antigenic *Borrelia* proteins associated with Lyme borreliosis, including already known and published protective antigens. Using several *in vitro* assays, we narrowed down the selection of vaccine candidates. These candidates plus some selected from the literature have been analyzed in our mouse protection models, challenging mice either subcutaneously or using laboratory infected ticks. From these initial studies, we have identified one promising candidate that shows some heterogeneity between different *Borrelia* strains. Therefore, we are investigating a multi-valent vaccine composition to achieve a satisfactory theoretical coverage against the different *Borrelia* species causing Lyme borreliosis in Europe.

B010 (D027)

Dementia-like syndromes in patients with Lyme neuroborreliosis

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Objective: To evaluate the frequency and characteristics of dementia-like syndromes in adult patients with Lyme neuroborreliosis (LNB).

Methods: A retrospective single-center study at the neurological and psychiatric departments of a tertiary health facility over a period of 9 years. Diagnostic classification of LNB was established according to EFNS guidelines (1). All patients with dementia-like syndromes and LNB showed pleocytoisis and intrathecal *Borrelia burgdorferi*-specific antibody synthesis in their cerebrospinal fluid. They were diagnosed and treated by one or more of the authors.

Results: Forty-six patients with definite LNB were identified. The vast majority of patients ($n = 40$) were diagnosed with Banwarth's syndrome (BS), a well-characterized painful radiculoneuritis. Subacute meningoencephalitis ($n = 1$), facial palsy without other symptoms of BS ($n = 1$) and acrodermatitis chronica atrophicans-associated neuropathy ($n = 1$) were other rare diagnoses. Three patients (6.5%) at the age of 71, 77, and 80, however, presented with a predominant dementia-like syndrome (MMSE 17, 22, and 16, respectively). None of them showed signs or symptoms of BS. Disease duration prior to admission was 3, 6, and 12 months. Their initial diagnosis was either Alzheimer disease or normal pressure hydrocephalus. All the three patients had severe deficits in their episiodic memory, disorientation ($n = 3$), acalculia ($n = 2$), and agraphia ($n = 2$) favoring a diagnosis of dementia. Rapid disease progression in combination with weight loss ($n = 3$), gait disturbance ($n = 3$), tremor ($n = 2$), urinary incontinence ($n = 2$), headache and vomiting ($n = 2$), and predominant attention deficits ($n = 3$), especially, were reasons to question the diagnosis of primary dementia. Complete remission or significant improvement of the dementia-like syndrome was achieved in all patients with a single course of 2 g ceftriaxone i.v. daily over 2–4 weeks. There were no relapses.

Conclusion: Dementia-like syndromes as predominant manifestation of LNB are rare. They made up for 6.5% of adult patients with LNB in our hospital population. In elderly patients, they may be misdiagnosed as primary dementia or as idiopathic normal pressure hydrocephalus. Therapeutic response to a 2- to 4-weeks course on ceftriaxone was excellent.

Reference:

1. Mygland A, Ljøstad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I, et al. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. *Eur J Neurol* (2010) **17**:8–16.

B011 (D034)

Bring back Lymerix!

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Lymerix is the only mode of intervention for which there is peer reviewed evidence for efficacy in reducing risk of the population level. It was withdrawn from the market due to financial considerations but those considerations have changed with the greatly expanded population at risk. Lymerix was safe and effective and is a tool of public health utility that could be used until second generation vaccines are developed, tested, and approved. I will review the reasons for reviving Lymerix, and what it would take to do so.

B012 (D039)

The immunogenic properties of candidate DNA vaccines against CCHF

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Crimean-Congo hemorrhagic fever virus (CCHFV) is viral zoonotic agent distributed widely in over 30 countries in Eurasia and Africa, causes severe disease in humans with a fatality rate of approximately 30%. The causative agent is a tick-borne virus, belonging to the family Bunyaviridae, genus *Nairovirus* that is transmitted to humans through infected tick bites, or by direct contact with viremic humans or animals. The epidemiology of CCHF reflects the geographic distribution of *Ixodid* ticks, belonging to the genus *Hyalomma*. The number of reported cases has increased in recent years, possibly due to climatic change and human perturbations of biocenoses that may have led to the migration of tick vectors. There is currently no commercially available vaccine for CCHF. Therefore, the aim of this study was to test the immunogenic properties of two candidate DNA-vaccines against CCHFV carrying the genes of structural proteins, plasmids pV3N, and pV3GN, obtained earlier in our organization. To study the immune response was performed twice by intramuscular immunization of mice of each of the plasmids and their mixture. In our study, cellular (methods ELISPOT and cell proliferation assay) and humoral (IEA) immune response were evaluated. Antigen-specific antibodies were detected in all experimental groups, except for the control group, after immunization. Numbers of cytokine-secreted cells were measured in commercial ELISPOT kits. The obtained results demonstrate that the immune response was induced in the T-cell type. The positive value for antigen-specific proliferation cultures of splenocytes stimulated *in vitro* with antigen of CCHFV was seen for group immunized mixture pV3N and pV3GN by the end of the observation. Our results show that immunization of mice plasmids pV3N, pV3GN, and their mixture leads to the formation of cellular and humoral immune response, and the use of a mixture of plasmids is the most promising as a candidate vaccine.

B013 (D041)

Cutaneous lymphoproliferative diseases associated with *Borrelia burgdorferi* in patients with malignant B cell lymphoma

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We here report on three patients with malignant B cell lymphoma who show *Borrelia burgdorferi* (Bb) associated cutaneous lymphoproliferative diseases responding to antibiotic therapy. High anti-Bb-IgG antibodies in all cases, detection of Bb by PCR in two (one of which also by culture) combined with substantial regression after antibiotic therapy argues for an important yet undefined role of Bb in pathogenesis of such rare manifestations. Case 1: a 58-year-old female patient with chronic lymphatic B cell leukemia (B-CLL) since 5 years developed cutaneous nodular leukemic infiltrates (nose, ear, both nipples), and blurry redness and edema of the face. Skin biopsy revealed B-CLL infiltrates. Bb serology showed strong IgG reactivity [immunoblot: p83, p58, p41, p21, DpbA, VlsE, (p43, p30,OspC)] and Bb *sensu stricto* was detected by PCR and cultivation from several skin biopsies. After ceftriaxone therapy of 2 g for 21 days, nodular skin infiltrates improved remarkably, but Bb DNA remained detectably. After a second 21 days course of ceftriaxone, the remaining skin infiltrates resolved and Bb DNA was not detectable afterward. B-CLL remains stable since 2 years. Case 2: a 64-year-old male patient was treated for intra-abdominal follicular lymphoma (three cycles of CHOP followed by eight cycles Rituximab as maintenance therapy) with good response. Ten months after start of therapy, he developed erythematous cutaneous infiltrates (face, ears, and dorsal hands) and complained about double vision, paresthesias, and diminished muscular power. Histology showed an interstitial granulomatous dermatitis with a dense lympho-histiocytic infiltrate with complete depletion of CD 20+ lymphocytes. *Borrelia* serology revealed strong IgG reactivity (immunoblot: p83, p58, p43, p41, OspC, Osp17, DbpA, p14, VlsE). PCR from skin biopsies (ear lobes, forehead) revealed DNA from both Bb s.s. and *Borrelia afzelii* as shown by RFLP. The patient recalled a tick bite with an insufficiently treated (7 days Doxycylin 100 mg) Erythema migrans in 1997. Skin infiltrates, musculoskeletal pains, and visual symptoms disappeared after ceftriaxone 2 g for 21 days. The retroperitoneal lymphoma is in complete remission since 2 years. Case 3: a 76-year-old male patient was treated for Non-Hodgkin Lymphoma with paraproteinemia with two cycles of Fludara and four cycles of Fludara/ Rituximab. During the last cycle, he developed erythematous infiltrates in his face and lower legs, looking very similar to the second patient. *Borrelia* serology showed very high IgG antibodies. He recalled a large erythema after tick bite 3 years ago, without antibiotic treatment. Skin infiltrates responded very well to ceftriaxon 2 g for 21 days.

B014 (D051)

Effectiveness of recombinant OSP-A Lyme vaccine in clinical practice

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Background: In 1998, a recombinant OSP-A vaccine (LYMErixTM) for Lyme disease (LD) was approved in the U.S. for use in persons aged 15–70 years. A pre-licensure randomized clinical trial using strict diagnostic criteria indicated an efficacy of 49–76%, depending on the number of doses received. In 2002, the manufacturer voluntary discontinued production of this vaccine. There has been no direct assessment of the effectiveness of this vaccine as it was used in clinical practice.

Objective: To assess the effectiveness of Lyme vaccine in Connecticut.

Design/Methods: We conducted a matched case–control study (up to two controls per case) of the effectiveness of Lyme vaccine. Cases of LD were identified from an active surveillance system of the CT Department of Public Health. Cases were classified as: definite LD – cases that met the national surveillance case definition for LD; Possible LD – cases that met most criteria but were missing some information (e.g., size of the erythema migrans rash); unlikely to have LD – all others. Controls were persons without LD selected using sequential digit dialing and were matched to the cases by age and area of residence. All medical records were reviewed to determine vaccination status and pertinent medical information. The effectiveness of Lyme vaccine was estimated from the matched odds ratios.

Results: We enrolled 869 age-eligible persons (median age: 48 years) reported to have Lyme disease from 2000 to 2003 (cases) and 1128 matched controls. Based on clinical and laboratory data, 64% of cases had definite LD, 14% had possible LD, and 18% were unlikely to have LD (4% had incomplete information). At least one matched control was enrolled for 709 cases (82%). Overall, 44/709 cases (6%) and 73/1128 controls (6%) had received at least one dose of Lyme vaccine. The vaccine's overall effectiveness was 1% (95% CI: -47 to 34%), P = NS. The vaccine was 37% effective (95% CI: -7.3 to 63%; $P = 0.08$) against definite cases of LD and 0% effective (95% CI: -422 to -12%; $P = 0.02$) for cases unlikely to have LD. However, the effectiveness of the vaccine for definite cases of LD who had received at least three doses of the vaccine was 58% (95% CI: 15–79%), $P = 0.02$. This estimate was unchanged after adjusting for potential confounders.

Conclusion: We conclude that the effectiveness of Lyme vaccine was poor in preventing all reported cases of LD, but that it was effective in preventing definite cases of LD after three doses of the vaccine. Misclassification of cases of LD is common and can affect the assessment of effectiveness of Lyme vaccine in clinical practice.

B015 (D052)

Clinical characteristics associated with *Borrelia burgdorferi* sensu lato skin culture results in patients with erythema migrans

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Background: The diagnosis of erythema migrans (EM) is primarily clinical, but the most reliable diagnosis also includes the isolation of *Borreliae* from the skin lesion. It is not yet clear how the clinical features in EM patients with a positive *Borrelia* skin culture result differ from those in whom isolation of *Borrelia* from skin was not successful.

Methods: To gain insight into predictors for a positive *Borrelia* EM skin culture, we assessed the differences in basic demographic, epidemiologic, and clinical data in 608 culture-positive patients and 501 culture-negative adult patients with the clinical diagnosis of solitary EM according to Slovenian case definition criteria for EM.

Results: In comparison to patients with negative skin culture result, those with a positive *Borrelia* spp. culture were older (OR = 1.10, $p = 0.03$), had more often an interval >2 days between tick bite and the onset of EM (OR = 2.74, $p < 0.001$), were more likely to have an EM diameter ³5 cm (OR = 3.00, $p = 0.02$), and the lesion was more often located on the extremities (OR = 1.64; $p = 0.001$). In contrast, a positive culture result was not associated with the recollection of the tick bite at the site of EM, the expansion of skin lesions, or central clearing. Restricted cubic splines and univariate logistic regression analyses revealed that the probability of a positive culture increased markedly with rising size of the largest diameter of the lesion up to ~15 cm and decreased slowly thereafter. A patient with a 15-cm EM lesion had almost threefold greater odds for a positive skin culture than patients with a 5-cm lesion. Multiple regression analysis showed that patients with an interval of at least 2 days between tick bite and the onset of EM had threefold greater odds for EM positivity than patients who reported tick bite but had no time interval between the bite and the onset of the lesion.

Conclusion: Several clinical characteristics, including some of those used in EM case definitions, were associated with a positive *Borrelia* spp. skin culture result. The findings are limited to adult European patients with solitary EM which was caused predominantly by *Borrelia afzelii*, and may not be applicable for other causative agents or different clinical situations.

B016 (P004)

Quantitative detection of *Borrelia burgdorferi sensu lato* in skin samples of erythema migrans patients: correlation of results with clinical findings

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Objective: Limited data are available on the correlation of *Borrelia* burden detected in skin biopsies retrieved from patients with erythema migrans (EM) and the course of disease and treatment outcome. The aim of the presented study was to determine if *Borrelia* burden in skin biopsies from EM lesions correlates with pretreatment clinical course of the disease and treatment outcome.

Methods: Skin biopsies taken from 121 adult patients with solitary EM lesions were tested by culture and quantitative PCR (qPCR) for the presence of *Borrelia burgdorferi sensu lato*. In addition, epidemiological, clinical, and microbiological evaluations were performed at baseline, as well as 14 days, 2, 6, and 12 months after commencing treatment with either amoxicillin or cefuroxime axetil.

Results: A total of 94 from 121 (77.7%) samples were positive for *B. burgdorferi* sensu lato by qPCR testing and 65 of 118 (55.1%) biopsies yielded a positive culture, of which 96.8% were typed as *Borrelia afzelii* and 3.2% as *Borrelia garinii*. Based on the qPCR data, the median number of spirochetes per biopsy was significantly higher in culture-positive versus culture-negative specimens. The association between *Borrelia* burden in skin biopsies and the pretreatment course of the disease (represented by demographic, epidemiological, clinical or laboratory characteristics) as well as treatment outcome (primarily assessed as percentage of patients with complete clinical response) was found not to be statistically significant.

Conclusion: Based on these results, we assume that neither *Borrelia* skin culture results nor *Borrelia* burden in skin biopsy specimens significantly influence the pretreatment course of the disease or treatment outcome in EM patients.

B017 (P016)

Tick-mediated *Borrelia burgdorferi* infection of nonhuman primates for assessment of antibiotic efficacy

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Introduction: With over 30,000 new cases reported annually, Lyme disease is the most common tick-borne infection in North America. The causative agent, *Borrelia burgdorferi*, can chronically infect humans, causing rash, arthritis, carditis, and neurological dysfunction. A proportion of Lyme disease patients experience symptoms after antibiotic treatment and the etiology of those symptoms are a matter of debate. Our prior results have demonstrated that *B. burgdorferi* spirochetes, administered by injection, could persist in nonhuman primates (NHP) after antibiotic therapy. We aimed to test this again following tick-mediated infection.

Methods: Ten macaques were tick-inoculated with *B. burgdorferi* and five were treated 4 months later with a 28-day course of doxycycline. Assessment for infection includes serology and xenodiagnosis.

Results: All animals developed mild erythema at the sites of the tick bites, but only one developed a bona fide erythema migrans lesion. Months later, ticks fed upon monkeys for xenodiagnosis was efficient, but generated local papular lesions, indicating a moderate level of anti-tick immunity. We have begun the assessment of xenodiagnostic ticks for infection and the comprehensive serological analysis.

Conclusion: Nonhuman primates exhibit variable local responses to tick-feeding and divergent antibody responses following infection with the identical strain of *B. burgdorferi*. A better understanding of diverse responses in an outbred population may help to improve the diagnosis of Lyme disease.

B018 (P022)

Immune profiling in Lyme disease: serum chemokines as markers for acute infection and clinical outcomes

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Lyme disease is an immune-mediated inflammatory disease initiated by infection with the tickborne spirochete *Borrelia burgdorferi*. Chemokines are key effector molecules that orchestrate the migration of innate and adaptive immune cells and direct them to sites of tissue injury and inflammation. We have conducted a 2-year prospective cohort study of patients with early, untreated Lyme disease from a mid-Atlantic suburban population. Patients were seen at seven study visits between time of diagnosis (acute disease) and 2 years post-treatment follow-up. We have measured the levels of key chemokines in serum samples derived from a cohort of patients with early Lyme disease as well as age- and gender-matched controls. In particular, the chemokines CXCL9 (MIG) and CXCL10 (IP-10) are increased during early acute Lyme disease ($p < 0.0005$). Following antibiotic treatment, the levels of CXCL9 and CXCL10 are reduced, and remained low through 2 years of follow-up. Other chemokines principally involved in the movement of innate immune cells (ex.CXCL8/IL-8) were not significantly altered in acute Lyme disease. Consistent with the role of CXCL9/CXCL10 in attracting immune T cells to the site of infection, CXCR3+ expressing CD4 and CD8 T cells are reduced in the blood of early acute Lyme disease ($p = 0.003$ and $p = 0.046$, respectively) and the extent of the decrease correlated with chemokine levels. The levels of CXCL9/10 did not relate to the size or number of skin lesions, but surprisingly high levels of serum CXCL9/CXCL10 were associated with elevated liver enzymes levels ($p = 0.016$). In addition, acute Lyme patients who went on to develop Post Treatment Lyme Disease Syndrome (PTLDS) do not display decreased levels of CXCR3+ T cells during acute disease. Collectively, these results indicate that the levels of chemokines and chemokine receptor expression level patterns may prove to be informative biomarkers for Lyme disease and predicative of disease outcomes.

B019 (P042)

Elevated IFNα activity in patients with a history of Lyme disease and persistent cognitive deficits

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Following antibiotic treatment for Lyme disease, some patients report persistent or relapsing symptoms of pain, fatigue, and/or cognitive deficits. Factors other than active infection, including immune abnormalities, have been suggested, but few clues regarding mechanism have emerged. IFNα is a cytokine that is produced in response to inflammation by a variety of cells. Several studies have shown a correlation between increased IFN activity and neurocognitive impairment in humans and in animal models. We carried out an analysis of serum IFNα activity in 19 *Borrelia burgdorferi* seropositive patients with a history of Lyme disease and persistent objective memory impairment. Control specimens included sera from 11 borrelial seropositive individuals with a history of Lyme disease who were symptom free, as well as 20 healthy individuals without serologic evidence or history of Lyme disease. IFNα activity was measured by detection of serum-induced changes in specific target genes, using a functional cell-based assay and quantitative real-time PCR. Sera from the patient cohort induced significantly higher expression of IFIT1 and IFI44 target genes than those from healthy controls, indicating increased IFNα activity. The increase in IFNα activity yields novel clues regarding the mechanism contributing to the ongoing neuropsychiatric symptoms in patients with a history of Lyme borreliosis.

B020 (P043)

Clinical and histological disease patterns in the skin in Lyme borreliosis

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Lyme borreliosis can behave like a chameleon presenting with different clinical disease spectra similar to syphilis and Lupus erythematosus. The classical clinical symptoms erythema migrans (EM), *Borrelia* lymphocytoma (BL), and acrodermatitis chronica atrophicans (ACA) are clearly defined. Histologically, EM shows a perivascular dermatitis although different clinical variants can be observed as a homogenous erythema as seen in *Borrelia garinii* infection or anular lesions mostly observed after *Borrelia afzelii* infection. In clinically resolving EM lesions, papules and nodules can arise, histologically characterized as granulomas, lymphocytoma, interstitial myositis of the arrector pili muscle or as an infiltrative lymphadenosis benigna cutis. BL presents as a polyclonal B- and T-cell proliferation and in ACA a T-cell-mediated infiltrate with plasma cells is typical, however, leading to severe damage of all skin structures including the epidermis, the collageneous, and elastic tissue in the latter disease. A similar tissue reaction like in ACA can also occur as an acute event, similar to early morphea, with collagen degeneration. In chronic *Borrelia* infection skin sclerosis, fibroid nodules and anetodermas are observed. EM can also present a Köbner phenomenon for the outbreak of psoriasis, in *B. afzelii*-induced morphea lesions, reversible Churg-Strauss granulomas were observed after contact with mineral wool. Granuloma anulare like lesions with molecular detection of *Borrelia burgdorferi* were described. An acute diffuse reversible sclerosis of the trunk was seen in a patient with Raynaud's phenomenon. Cutaneous calcinosis in EM area was observed in a patient with dermatomyositis. *Borrelia* can induce partly reversible malignant proliferations including a *B. afzelii*-induced marginal cell lymphoma. In summary, *B. burgdorferi* can induce local tissue destructions leading to atrophy or sclerosis, granulomatous tissue reactions and benign and malign lymphoproliferative disorders.

B021 (P044)

Disorders of blood coagulation in patients with acute *Borrelia miyamotoi* infection

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Introduction: The borreliosis caused by *Borrelia miyamotoi* (Bmt) is a new human infection first described in Russia. In addition to high fever (38°C), fatigue, headache, and other flu-like symptoms, about 60% of Bmt patients have signs of mild or moderate organ dysfunction, including the liver, kidney, heart, and lung. *Borrelia* secretes no known toxins, so we hypothesized that disorders of blood microcirculation might be responsible, at least in part for organ dysfunction.

Methods: Twenty-four patients with Lyme disease (LD) and 28 Bmt patients treated in Izhevsk City Hospital (Russia) in 2010–2011 were enrolled in the study. Laboratory confirmation of acute LD and Bmt cases was performed according to a previously published report (Platonov et al., 2011). On admission, the Bmt patients were subjected to standard coagulation testing. In addition, the blood microcirculation was studied by slit lamp microscopy.

Results: Most of the 28 Bmt patients had some coagulation pathway abnormalities: activated partial thromboplastin time <24 s (54%); international normalized ratio (INR) <0.7 (36%) and INR >1.3 (18%); thrombin time (TT) <13 s (25%) and TT >18 s (18%); antithrombin III level £75% of normal value (50%); fibrinogen level >4 g/L (26%); euglobulin lysis time greater than the upper normal limit for age (21%); and the presence of d-dimers (43%). Platelet counts were <150,000/mL of blood in 12 patients (43%). All the Bmt patients had at least one abnormal coagulation parameter of the eight that were tested. Eighteen (64%) of the Bmt patients had marked coagulopathy with three to six abnormal laboratory findings. In contrast, all the eight parameters were normal in 17 (71%) patients with LD. The other seven LD patients had only one or two abnormal coagulation parameters ($p < 0.001$ in comparison with Bmt patients). Microscopic examination of eye capillary blood flow revealed pathological findings that included aggregates of erythrocytes and platelets and obstructed and/or sinuous capillaries in 22 (79%) of the Bmt patients, but none of the LD patients. A total of 14 Bmt patients had both coagulation and microcirculatory abnormalities. Eleven of them also had transient signs of organ dysfunction.

Conclusion: Disorders of blood microcirculation in Bmt patients may contribute to organ dysfunction.

B022 (P047)

Cerebral sequestration associated with neurovirulence is reduced after serial *in vivo* passage of *Babesia bovis*

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Severe neurological signs develop during acute infection by virulent strains of *Babesia bovis* in cattle, similar to those produced in humans suffering from Plasmodium falciparum malaria. Neurovirulence is postulated to be associated with cytoadherence of infected erythrocytes to cerebral capillary endothelium and subsequent capillary sequestration. Serial passage of virulent strains in cattle results in attenuated derivatives that do not cause neurologic disease. We evaluated *in vivo* whether attenuated strains derived by serial passage lose the property of cerebral sequestration by examining brain biopsies at different times during acute infection. We then tested whether subsequent tick passage would result in reversion to neurovirulence with an associated change in cytoadherence ability. Cerebral biopsies of spleen intact animals inoculated intravenously with a parental virulent or derived attenuated strain of *B. bovis* were evaluated for capillary sequestration at two different time points: (1) at the onset of clinical babesiosis; and (2) during severe clinical disease. In animals infected with the virulent parental strain, there was a significant increase in sequestration between the first and second biopsy time points. The passaged derivative strain, while still capable of sequestration at a significantly reduced level, did not change between the first and second sampling. Necropsy examination confirmed the second time point findings and demonstrated that sequestration identified at necropsy reflects pathologic changes occurring in live animals. Tick passage of virulent *B. bovis* did not alter the clinical or pathologic phenotype, resulting in acute, severe babesiosis with cerebral sequestration in calves exposed to infected larvae. However, animals exposed to larvae infected with the passaged derivative strain did not develop acute babesiosis, nor was any sequestration observed in cerebral capillaries. Interestingly, although sequestration in the brain did not occur following tick passage of the attenuated derivative strain, qPCR analysis of multiple tissues showed no significant differences in the level of parasitemia between animals infected with the virulent or attenuated derivative. Serial *in vivo* passage of this virulent *B. bovis* strain in splenectomized animals results in a significant decrease in sequestration and neurovirulence when compared to the parental strain. In addition, not only does the derived attenuated strain maintain tick transmissibility, it does not revert to virulence following tick passage. The lack of clinical signs and sequestration after passage within the tick, despite similar parasite tissue levels, is striking and suggests that there are other factors that mediate cerebral sequestration leading to neurovirulence.

B023 (EC034)

Symptomatology and clinical picture of post-treatment Lyme disease syndrome

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Introduction: After antibiotic treatment for Lyme disease, a subset of individuals report persistent symptoms associated with functional decline, termed Post-Treatment Lyme Disease Syndrome (PTLDS). One challenge in rectifying the PTLDS literature is the lack of consistent methodology for measuring the symptoms of PTLDS. The current study incorporates structured, standardized instruments to capture the symptom picture of individuals with early Lyme disease.

Methods: Sixty-six patients with clinically diagnosed untreated Lyme disease with active erythema migrans were enrolled in a prospective cohort study. Patients were treated with 3 weeks of doxycycline and followed for 6 months. At each visit, a physical examination, interval history, clinical symptoms, and self-administered questionnaires were performed. Additionally, at visits 2 and 3, standardized neurocognitive testing was completed. At 6 months post-treatment, an operationalized definition based on the IDSA criteria for PTLDS was used to group these patients according to symptom persistence and SF-36 health function scores. Eight patients met criteria for PTLDS (PTLDS+). Twenty-six matched controls were also evaluated at two visits 6 months apart.

Results: At the time of diagnosis (pretreatment), both the PTLDS+ and PTLDS- groups differed from controls on symptoms of pain ($p < 0.0005$) and somatic depressive symptoms ($p < 0.05$). However, there were no statistically significant group differences on fatigue or affective depressive symptoms. Immediately post-treatment, the PTLDS+ group differed from both the PTLDS- and controls on symptoms of pain ($p < 0.0005$), and somatic depressive symptoms ($p < 0.01$), but not on symptoms of fatigue or affective depressive symptoms. Finally, at 6 months post-treatment, the PTLDS+ group was statistically significantly different from the PTLDS- group and controls on measures of fatigue ($p < 0.05$), pain ($p < 0.01$), somatic depressive ($p < 0.0005$), and affective depressive symptoms ($p < 0.01$). On the neurocognitive testing, we found no significant differences in cases and controls.

Conclusion: There is a distinct pattern of symptoms for individuals who meet criteria for PTLDS at 6 months post-treatment that can distinguish these individuals from the PTLDS- group and controls. Specifically, PTLDS+ shows a pattern of pain and somatic depressive symptoms at 4 weeks post-treatment. And then by 6 months post-treatment, PTLDS+ have notable fatigue, affective and somatic pain, and affective and somatic depressive symptoms that are distinct from PTLDS- and controls. Interestingly, there is no evidence of true persistent cognitive decline or major depression in the PTLDS+ or PTLDS- groups.

B024

European Lyme disease transmission model: an essential tool to study vaccine candidates against human borreliosis

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Lyme disease is an emerging vector-borne disease of temperate climates with a concurrent distribution spanning North America and Eurasia. It is caused by *Borrelia* species collectively known as *Borrelia burgdorferi* sensu lato, primarily transmitted by *Ixodes* spp. ticks. An intensive effort has been put toward elucidation the host–pathogen interactions during *Borrelia* infection. The main aim of this endeavor was to find out factors that could be used as vaccinogens to prevent *Borrelia* transmission. Better understanding of the mechanisms of Lyme disease transmission can be approached by using laboratory animal models. Present transmission models testing the *Borrelia* vaccine candidates employ *Ixodes scapularis* nymphs – *B. burgdorferi* sensu stricto – mice system. Our aim was to develop a transmission model concerning main European tick species *Ixodes ricinus* and European Lyme disease agents (*Borrelia afzelii*, *Borrelia garinii*, *B. burgdorferi* sensu stricto) to test candidate tick antigens studied and characterized in our laboratory. Infectious, low-passage *B. burgdorferii* isolates were grown in a culture and injected subcutaneously into mice. Noninfected *I. ricinus* larvae were fed on *Borrelia* infected mice to generate infected nymphs. Presence of *B. burgdorferi* spirochetes within the tick nymphs was tested using nested PCR amplifying a part of 16S–23S intergenic region. The infection rates in nymphs were evaluated by quantitative real-time PCR. To examine *Borrelia* infection in mice, biopsies from different organs (ear, skin, heart, urinary bladder, and joint) were analyzed. Biopsies were first screened using nested PCR protocol to detect *Borrelia* DNA, and positive samples were further quantified using quantitative real-time PCR. Our results showed that urinary bladder is the most reliable tissue for *Borrelia* detection during persistent murine infection (at least 4 weeks post infection). However, additional examination of heart muscle and/or joints is recommended for precise detection of spirochetal infection in mice. Optimized transmission model was further employed to test tick immune genes influencing *Borrelia* dissemination within the tick. Current knowledge of tick immunity in relation to *Borrelia* infection will be presented.

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B025

The first case of meningoencephalitis by the relapsing fever spirochete *Borrelia miyamotoi* in Europe

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We here describe a case of meningoencephalitis in an immunocompromised patient with a history of non-Hodgkin lymphoma and rituximab therapy caused by the relapsing fever spirochete *Borrelia miyamotoi*. A 70-year-old man had experienced many tick-bites in the Netherlands while hiking nearby his recreational house in the dunes. He presented with cognitive slowing, memory deficits, and a disturbed gait, which had gradually developed over several months and were progressive over the last few weeks prior to presentation. Upon neurological examination, there was a distinct bradyphrenia and the patient scored 26 of 30 points on the mini mental state examination. Vital signs were normal and body temperature was 36.4°C. Cranial MRI showed no abnormalities, but repeated lumbar punctures showed cerebrospinal fluid (CSF) pleocytosis. Extensive microbiological, pathological, and haematological diagnostic testing initially failed to reveal the cause of this chronic meningitis. In light of the frequent tick exposure and an obvious relation in time with recent tick bites, he was treated with ceftriaxone intravenously for 2 weeks for a possible Lyme neuroborreliosis. Over a period of several weeks, the patient fully recovered. Supported by the recent evidence of the presence of *B. miyamotoi* in *Ixodes ricinus* ticks across Europe and a recent case report in the *NEJM* on *B. miyamotoi*-induced meningoencephalitis in a similar patient in the USA, we retrospectively considered *B. miyamotoi* as the causative agent. Indeed, in stored pre-treatment cerebrospinal fluid (CSF), we showed the direct presence of motile spirochetes by dark field microscopy. In addition, a 16S rDNA pan-relapsing fever *Borrelia* quantitative (q)PCR and a qPCR targeting the *B. miyamotoi flagellin* gene proved to be positive in two separate pre-treatment CSF samples and a pre-treatment blood sample. Interestingly, 2.2% of 352 questing *I. ricinus* nymphal ticks from the vicinity of the patient's recreational house were positive for *B. miyamotoi* by qPCR. Amplification and sequencing of the *glpQ* and *p66* genes confirmed *B. miyamotoi* as the causative agent and revealed 100% identical sequences in ticks and the patient's clinical samples. ELISA and Western blot did not reveal anti-GlpQ antibodies in blood and CSF. This case represents the second documented case of *B. miyamotoi*-induced meningoencephalitis in the world and the first in Europe. It underscores that physicians worldwide confronted with immunocompromised patients from *Ixodes* tick-endemic areas with a meningoencephalitis should consider *B. miyamotoi* as a potential causative agent and should be aware that regular diagnostic tests for *Borrelia burgdorferi* will most likely overlook this diagnosis. Whether *B. miyamotoi* is also able to cause neurological symptoms in immunocompetent patients requires further investigation.

B026 (D003)

Serum antibody responses of patients with southern tick-associated rash illness (STARI)

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STARI is a provisional name of an illness of unknown etiology associated with the bite of lone star ticks (*Amblyomma americanum*), a species that is most common in the south and southeastern United States. To study the cause of STARI, patients were enrolled in an IRB-approved study to collect clinical specimens including serum. The enrollment criteria were the presence of a physician-diagnosed erythema migrans (EM)-like rash (³5 cm) and possible exposure to ticks in the southern U.S. or, if outside this region, an attached lone star tick at the rash site. Here, we evaluated STARI patients for evidence of exposure to *Borrelia burgdorferi* sensu lato or to *Borrelia lonestari* by serology. Antibody responses (IgM and IgG) in acute- and convalescent-phase (A/C) serum samples of STARI patients were evaluated by ELISA against antigens from 10 strains of *Borrelia*. *B. burgdorferi* sensu lato whole cell antigens were prepared from representative strains isolated from ticks or rodents in the southern U.S.: *Borrelia americana* SCW-30E, *B. americana* SCW-33, *Borrelia andersonii* SI-10, *Borrelia bissettii* FD-1, *B. burgdorferi* MI-4, *B.burgdorferi* SCW-6, and *Borrelia carolinensis* SCW-14. Antibody responses to *B. lonestari* were evaluated using recombinant glycerophosphodiester-phosphodiesterase (GlpQ) antigen from *B. lonestari* and a surrogate whole-cell antigen from *Borrelia miyamotoi* FR64b (Japan), the species most closely related to *B. lonestari* that is available in pure culture. In addition, standard two-tiered serology for Lyme disease was performed using antigens from strain B31. Serum samples from two types of control populations were studied using the same experimental ELISAs: healthy blood donors and early Lyme disease (EM) patients. Serum from healthy blood donors was obtained at two points in time separated by 24–37 days. Paired A/C serum samples from patients with culture-confirmed Lyme disease were obtained from the CDC reference collection. ELISA OD differences between the first and second sample were calculated for each individual in the three populations. To compare the distributions of ELISA OD differences among the three populations, an ordered heterogeneity test will be employed. The test orders the groups according to mean ranks and correlates the observed ranks to the possible expected ranks. Preliminary analysis compared the distributions of OD differences for antibody responses of STARI patients and healthy blood donors to GlpQ (*B. lonestari*). Results from the two-sample Kolmogorov–Smirnov test ($p = 0.83$) and equivalence test of distribution means ($p < 0.001$) indicate that responses of these two populations to GlpQ are the same.

B027 (D012)

Comparison of two beads immunoassays for serological diagnosis of Lyme borreliosis

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Objective: The two beads immunoassays were evaluated and compared for performance of the serological diagnosis of Lyme borreliosis

Methods: Multianalyt™ *Borrelia burgdorferi* IgG and IgM (Virion/Serion, Würzburg) on BD FacsCanto II (Becton Dickinson) and Multimetrix *Borrelia* IgG and IgM (Progen, Heidelberg) assays on Luminex were evaluated and compared with our in-house Western blots. We performed both assays according to manufacturer's instructions. We evaluated sensitivity with a panel of sera from patients presenting an erythema migrans (EM) (n = 25), with early disseminated stage of neuroborreliosis (NB) (n = 30) confirmed by a specific intrathecale antibody synthesis, as well as with late disseminated stages (LD) arthritis or acrodermatitis chonica atrophicans (n = 20). Specificity was challenged with 80 sera from blood donors (DON) living around the city of La Chaux-de-Fonds, a low endemic area for Lyme borreliosis (JuraMountains).

Results: Specificity of IgM, IgG, and cumulative IgM or IgG (G + M) obtained for Multianalyt (MA) and Multimetrix (MM) were respectively 91.3, 88.8, and 82.5% for MA, and 83.8, 78.8, and 63.8% for MM. To compare, we obtained 92.5, 92.5, and 86.3% with our immunoblots. Included are true positive revealing seroprevalence in asymptomatic donors and a false-positive rate. The rates of equivocal results obtained in G + M were as high as 20% for MM and 11.3% for MA. For EM positive, results obtained for IgM, IgG and G + M were 68, 48, and 72% (MA) and 76, 68 and 88% (MM), respectively. For NB, they were 33.3, 80, and 83.3% (MA) and 66.7, 96.7, and 100% (MM), respectively. All LD tested were positive in IgG for both the assays. Equivocal results could be as high as 24 or 16% for IgG in EM, respectively, for MA and MM, but special focus was made on true positive results. These assays are considered as confirmatory tests and results should be positive or negative. Equivocal results are useless for confirmation.

Conclusion: Globally, the assays showed good to higher sensitivity than our confirmatory blots. However, we are concerned about the too low specificity that will generate too many false positive results on clinically poorly defined borreliosis cases. Assay evaluation in a country should be presented before commercialization to reflect local epidemiology and determine essential test values as seroprevalence, specificity, and sensitivity. Confirmatory assays for Lyme borreliosis are to be very specific (>95%) to be useful.

B028 (D014)

Study on the cross reactivity of anti-*Borrelia* and treponemal antibodies

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Due to the antigenic relationship of *Treponema* and *Borrelia*, false positive results in *Borrelia* antibody tests can occur in samples from syphilis patients. In Germany, there is an actual discussion about the need to introduce a mandatory syphilis testing for all positive *Borrelia* antibody findings, to rule out nonspecific results induced by treponemal antibodies. The relevance of this requirement was the topic of this study.

Samples: First, 50 selected samples with borrelial antibodies (panel 1) and 50 samples with treponemal antibodies (panel 2) were regardless of the results tested with all diagnostic tests used in this study. Following 85 samples from the syphilis routine laboratory (panel 3) were included in the study as well.

Tests: Liaison® *Borrelia* IgG CLIA (DiaSorin), recomWell *Borrelia* IgG/IgM-EIA (Mikrogen), Enzygnost® Lyme link Vlse IgG and Borreliosis IgM (Siemens), anti-*Borrelia* plus VlsE-ELISA IgG and anti-*Borrelia*-ELISA IgM (Euroimmun), *Borrelia* ViraStripe® IgG/IgM(Viramed), recomLine *Borrelia* IgG/IgM (Mikrogen), Architect Syphilis TPCMIA (Abbott), TPPA (Fujirebio), IgG- and 19S-IgM-FTA-ABS-Test, RPR (Biokit), Treponema + VDRL Virablot® IgG/IgM (Viramed), recomLine Treponema IgG/IgM (Mikrogen).

Results: Specificity of syphilis tests in 50 samples with *Borrelia* antibodies (panel 1): Architect Syphilis TP CMIA 100%, TPPA 98%, IgG-FTA-ABS 96%, 19S-IgM-FTA-ABS 100%, Treponema + VDRL Virablot IgG/IgM 100%, recomLine Treponema IgG/IgM 100%. For the 50 syphilis samples (panel 2), IgG antibodies against the borrelial antigens p83/100, p58, p43, p39, OspA, OspC, p18, p17 and p14 could not be detected. In addition, in the recomLine *Borrelia* IgG assay, all VlsE antibody findings were negative. Using the ViraStripe® *Borrelia* IgG test in six samples, antibodies against p30, and in two different samples VlsE antibodies, were demonstrated. The recomLine *Borrelia* IgM showed no false positive result. In the ViraStripe® *Borrelia* IgM, five samples showed OspC antibodies of questionable specificity. In more than 50% of the syphilis samples, IgG p41-antibodies with a slightly lower frequency but partially strong reactivity IgG-p41 antibodies were demonstrated. Specificity of *Borrelia* IgG-tests in 135 syphilis samples (panels 2 and 3) were as follows: recomWell *Borrelia* IgG ELISA 99.3%, LiaisonBorrelia IgG CLIA 98.5%, Enzygnost Lyme link VlsE IgG ELISA 100%, and anti-*Borrelia* + VlsE-ELISA IgG 64.7%. Specificity of *Borrelia* IgM tests in 135 syphilis samples (panels 2 and 3) were: recomWell *Borrelia* IgM ELISA 96.9%, Enzygnost Borreliosis IgM ELISA 95.5%, and anti-*Borrelia*-ELISA IgM 93.8%.

Conclusion: Samples positive for borrelial antibodies usually do not lead to false positive syphilis antibody findings. Inadequate specificity is a problem, when syphilis samples are tested with an assay based on *Borrelia* whole cell antigen preparations, but not when recombinant antigens are used or when in whole cell antigen assays a preabsorption step for the elimination of potential crossreacting antibodies is integrated. Using the two-tier diagnostic procedure, the specificity of the anti-*Borrelia* screening results is usually clarified. Therefore, an additional routine application of a syphilis test is not necessary. However, a routine syphilis testing without consent of the patient is also an ethical problem.

B029 (D021)

Relevance of quantitative determination of IGG antibodies against VlsE as an activity marker in the monitoring of treated Lyme borreliosis: a retrospective study

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Introduction: The first step in the serological diagnosis of Lyme borreliosis comprises a sensitive screening test containing a selected spectrum of native and/or recombinant antigens, including the highly specific recombinant VlsE. From the literature, it is well known that high levels of anti-VlsE antibodies in particular can be detected in the acute phase of a Lyme infection. The goal of our retrospective study was to systematically and quantitatively monitor anti-VlsE IgG antibodies before and after treatment using a specially adapted test system.

Methods: We used primarily a classic indirect ELISA from EUROIMMUN containing recombinant VlsE antigen from *Borrelia burgdorferi* sensu stricto for the quantitative *in vitro* determination of human IgG antibodies against the VlsE antigen. A special version of the test which is optimized for titer monitoring was also used. The titer level is measured in relative units (RU/ml). The detection limit of the test is 1 RU/ml and the cut-off is 16 RU/ml. Archived (–80°C) sera from patients with confirmed or suspected Lyme disease from our Lyme outpatient clinic were used as sample material.

Results: A significant drop in the titer of anti-VlsE antibodies could be detected as early as 6–8 weeks after successful treatment in all active chronic Lyme infections, including Lyme arthritis, acrodermatitis chronica atrophicans, acute neuroborreliosis, and other active chronic stages of Lyme infection. The decrease in anti-VlsE correlated with a reduction in clinical symptoms. The absence of an anti-VlsE titer virtually excludes a florid chronic Lyme infection (control panel of healthy individuals, $n = 105$). Strongly positive anti-VlsE values (>1000 RU/ml) in untreated patients are to 99% an indication of an active chronic Lyme infection. Some patients with erythema migrans (EM), in contrast, showed only low anti-VlsE values, which increased slowly and/or became negative again a short while after treatment.

Conclusion: Quantitative determination of anti-VlsE IgG is suitable – always under consideration of clinical symptoms – for confirmation of diagnosis and as an activity marker for monitoring patients with active chronic Lyme borreliosis before and after treatment.

B030 (D023)

CDC Lyme disease serum repository; collection, analysis, and availability

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In the United States, nationally recommended laboratory confirmation for Lyme borreliosis is based largely on assessment of antibody responses to infection with *Borrelia burgdorferi* sensu stricto. Development and evaluation of diagnostic tests for Lyme disease is dependent upon analysis of samples from well-documented patients representing a full spectrum of disease presentations and negative controls composed of likely-to-been countered healthy subjects and patients with alternate disease etiologies. Comparative test performance also depends on analysis of these types of samples and can be greatly strengthened by parallel sample set testing. In practice, however, access to such samples is often restricted to unique clinical settings, where these types of patients and controls are seen; well-documented and widely available patient sample sets are generally not available. In 2008, NIH and CDC committed to establishing such an archive for Lyme disease and between 2009 and 2013, a serum repository, collected through funded contracts, was built. Samples from over 400 US Lyme disease patients, healthy endemic and non-endemic subjects, and persons with other diseases that may overlap with those of Lyme disease in clinical presentation or antibody reactivity were collected. Non-Lyme disease patients included those with multiples sclerosis, fibromyalgia, infectious mononucleosis, severe periodontitis, syphilis, and rheumatoid arthritis. Patient documentation and inclusion targeted nationally standardized case definitions and laboratory support that avoided serological test outcome for Lyme disease wherever possible. Alternate proofs for Lyme disease included culture, PCR, clinical history, and epidemiological risk. Upon receipt by CDC, all samples were evaluated by a variety of Lyme disease serological tests including standardized two-tier testing. Performance in these tests is presented and highlights the strengths and weaknesses of currently available tests and test strategies as well as the utility and limitations of the described serum archive. Sera from well-documented Lyme disease patients and controls along with test results and clinical documentation are now available. Samples are provided in limited number and volume sets, coded or un-coded, for the purpose of Lyme disease serological test validation and research toward development of improved Lyme disease diagnostics and FDA clearance.

B031 (D024)

High incidence of atypical erythema migrans in PCR proven Lyme disease

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The ability to unequivocally diagnosis Lyme disease early is advantageous to the patient and the research field as it can provide a validated group for basic and clinical studies. The best diagnostic sign in early Lyme disease when it occurs, is a skin lesion, erythema migrans (EM). However, it may not occur or be recognized in 30% of cases. Among those that do occur, it has been reported that approximately 20–30% of EM rash may not display a classic bull's-eye, ring-within-a ring, appearance, fact that may be underappreciated. Atypical variants of EM have included a blue-red appearance and occasionally a vesicular central region 4.5. Other cases with annular lesions may have a differential of fixed drug eruptions, granuloma annulare, cellulitis, dermatophytosis, or systemic lupus erythematosus. We began to assess the incidence of atypical EM in cases in whom we could provide definitive microbiological proof of a *Borrelia burgdorferi* infection. We used an enhanced multilocus PCR assay for blood. In a set of 21 patients, we identified 10 PCR-positive cases with non-classic EM lesions and 4 PCR-positive subjects had classic ring-within-a ring target lesions. Thus among PCR+ cases (also met CDC criteria), 10/14 (71%) had a non-classic EM. All lesions were >5 cm and were categorized by an experienced panel including dermatologists who published in the field. Atypical presentations ranged from those close to classic EM to those resembling lesions more common in other conditions such as insect or spider bites and consequently prone to misdiagnosis. Separately in another 12 subjects from a different endemic area, we analyzed tick, blood, and skin for genotypes of *B. burgdorferi* that have been reported to disseminate and cause systemic disease. In no case, where blood was PCR, did we found a disseminating-OspC genotype. In summary, the occurrence of atypical EM is high enough that clinicians should consider Lyme disease in patients with endemic area exposure and a rash that is not the classic EM. (For presentation, we will show Photos and Figures.)

B032 (D029)

Development of an algorithm for rational *Borrelia* serology in a high endemic European area

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In an endemic high-risk area as the Åland Islands, with annual incidence of Lyme borreliosis of >1000/10^5, a rational use of diagnostic serology is most important. In this setting, with a high background seropositivity rate of 20–60%, serology is applied to rule out the presence of disseminated Lyme borreliosis, rather than for ruling-in the disease. High sensitivity assays are therefore necessary without reduction of specificity. A two-step serology is most important for preserving the specificity, but also costly. We have previously demonstrated that a combination of two ELISA assays may be useful for reduction of immunoblotting. We have studied 15,490 blood samples from a clinical material using C6 Lyme ELISA Kit (Immunetics, USA) and recomWell IgG (Mikrogen, Germany). Of these, we found positive recomWell in 54.3%, equivocal in 4.1% and negative in 41.6% while C6 antibodies was positive in 40.5%, equivocal in 2.9%, and negative in 57.8%. The difference in the positive fractions was 15% (95% CI 13.5–16.5) and highly significant $p < 0.0001$. In an aliquot of 3380 samples, we confirmed the ELISA results by IgG immunoblot, recomLine (Mikrogen, Germany). The confirmation of positive results in the C6-antibody test by standard immunoblotting against a panel of recombinant *Borrelia* antigens is not recommendable. The ELISA tests used did not perform identically in detecting *Borrelia*-specific antibodies and we find definite cases of disseminated LB with either test negative in a clinical material. An algorithm for rational, cost-saving serology for Lyme borreliosis will be presented. Evaluation of this algorithm during 1 year has shown a reduction of ELISA tests by 27% and immunoblots by 20%, which transforms into 26.8% cost reduction.

B033 (D030)

Evaluation of Mikrogen recombead *Borrelia*-specific IGG and IGM antibodies

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Introduction: This new technology where many biomarkers may be assessed simultaneously is promising compared to traditional immunoblots. The laboratory procedures are easier, more or less like an ELISA. Reading of results is quantitative and not based on manual and visual interpretation of bands of varying intensity on a narrow strip. This study presents: (1) result of a new quantitative multiplex assay (2) discussion of methods for scoring multivariate diagnostic data.

Materials: Serum samples from 49 patients with neuroborreliosis and 217 blood donors. The patients with neuroborreliosis were laboratory defined by pleocytosis and a positive antibody index.

Methods: Mikrogen recomBead *Borrelia* IgG and IgM on the Luminex platform, a technology that combines flow cytometry, microspheres, and laser technology. The same 13 different recombinant antigens were included in both the IgG and IgM assays.

Results: Qualitative results using the evaluation software supplied by the manufacturer 24 of 49 patients with Neuroborreliosis were found positive or borderline (Table 1) in IgM or IgG and thus the sensitivity was 48% (36–63). The sensitivity of IgG or IgM alone was 33 and 31%, respectively. The specificity measured in 217 blood donors was 97% (94–99) for IgG and 99.5% (97.4–100.0) for IgM. Quantitative analysis by creating a logistic regression model sensitivity of the assay was improved to 91% when choosing the specificity at 98%. Only IgG VlsE and IgM OspC had significant diagnostic importance. Most of the other included antigens had little if any reactivity in this group of patients with neuroborreliosis were slightly more reactive in the blood donor control group and were subtracted instead of added in the regression model. Technical performance was fairly easily implemented in the laboratory without any failed runs.

Discussion: The excellent results of the regression modeling needs to be confirmed in patients with other disease manifestations of Lyme borreliosis, especially patients with late disease like acrodermatitis or arthritis. Patients with late disease should be expected to have antibody reactivity to many of the antigens, which were “negative” in this group representative of patients with early disseminated Lyme disease. Also, geographical variations in patterns of seroreactivity to different antigens could be important for interpretative scoring of results. The results should be confirmed in an independent group of samples.

Conclusion: The scoring system provided by the manufacturer gave an inadequate sensitivity of 48%. However, combining the quantitative results in a logistic regression model and only the IgG VlsE and the IgM OscpC antigens had important discriminatory power. The optimized sensitivity was 91% at a specificity of 98%.

B034 (D031)

Determination of the CSF/serum antibody index for the diagnosis of Lyme neuroborreliosis: methodological considerations

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In the European case definitions for Lyme borreliosis (Stanek et al., 2011), it is stated that the analysis of paired serum and cerebrospinal fluid (CSF) samples obtained simultaneously is key to determining the specific CSF/serum antibody index (AI). A positive AI together with typical signs of inflammation in the CSF confirms a clinical diagnosis of Lyme neuroborreliosis. The purpose of the study was to asses the analytical performance and the strategy for choice of cutoff.

Methods: Results of 1510 index determinations, consecutive routine, from October 2001 to September 2010 were extracted from the laboratory information system. All determinations were performed by the Oxoid IDEIA Neuroborreliosis kit based on native flagella antigen. The main tool for assessing performance and choice of cutoff was the XY plot of the paired OD readings.

Results: About 1388 were negative and 122 (8.1%) were determined as index positive in IgG or IgM. The use of paired serum and spinal fluid samples has two advantages. (1) Increase of sensitivity, as the analytical cutoff may be lowered. In this assay, an OD of 0.150 seems well chosen to exclude the negative majority population. (2) Increase of specificity. Due to the blood-brain barrier, only patients with intrathecal antibody production may have a relatively increased reactivity compared to serum (the index principle). In this case, the calibrated index value of 0.3 also seems to be well chosen. These properties are shown in the XY plots together with the chosen cutoffs. Both a lower cutoff and an index cutoff are needed. For IgM, a cutoff for highly positive samples is also specified.

Discussion and Conclusion: This is an analytical exercise without clinical data, but nevertheless an important step in test evaluation before performing any clinical evaluation. The results show the practical and theoretical advantages of index determination. When the relative reactivity in serum and spinal fluid is considered for each patient, the variation between patients is filtered out. Thus, the serum for each patient is used as his own control. This allows for a low analytical cutoff to increase sensitivity and at the same time maintain a high specificity as patients with serum antibodies only are not reported positive. These results confirm the importance of using simultaneous determination of serum and spinal fluid when diagnosing Lyme neuroborreliosis.

B035 (D032)

Development of an IgG Western blot avidity assay to identify antigenic markers for the different stages of *Borrelia burgdorferi* infection in Scottish patients

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Immunoblot assays have been used to measure IgG antibody avidity to aid the timing of infection for a range of organisms, but not *Borrelia burgdorferi*. Serology is used to support a clinical diagnosis of Lyme borreliosis, but it is unnecessary in the use of antibiotics and may delay diagnosis of the actual cause of a patient's symptoms. The aim of this project was to develop an IgG Western blot avidity assay to identify potential markers for the different stages of Lyme borreliosis. An in-house IgG Western blot assay utilizing *B. burgdorferi* sensu stricto and *Borrelia afzelii* lysates was adapted by substituting the first wash step with 8 M urea/PBST washes. Densitometry was used to measure differences in band intensity between Western blot and avidity Western blot strips for each sample to assess antibody avidity to individual antigens. A decrease in band intensity of <75% (avidity index <25%) in the avidity strip was considered as a high-avidity antibody response. The optimized avidity assay was tested against 50 sera, including patients with confirmed early (n = 15) and late (n = 15) disease. Analysis of results identified potential markers of early (43 kDa antigen) and late Lyme borreliosis (46, 39, 32, 30, and 22 kDa antigens). These antigens were later characterized by 2D electrophoresis and mass spectrometry. Criteria to interpret avidity results based on the number of antigens (including the proposed early and late markers) detected with high avidity were devised to differentiate early, late, and past infection with *B. burgdorferi*. When prospective sera from 55 patients with clinically suspected Lyme borreliosis were tested with the Western blot avidity assay, the avidity results improved the overall result interpretation. The avidity interpretation in 28 patients was consistent with the clinical information provided and the Western blot results. A more definitive serological diagnosis of past infection was indicated in five patients. Twenty patients had insufficient clinical information to determine the stage of infection but the avidity assay indicated 8 early and 12 late Lyme borreliosis. The avidity results may have altered the management of five of the seven patients as they did not agree with the original result interpretation. The Western blot avidity assay has the potential to differentiate early, late, and past Lyme borreliosis, improving the management of Scottish patients. Detailed clinical follow-up and the investigation of the avidity time course of marker antigens will help confirm these initial findings.

B036 (D033)

Evaluation of SpiroFind, a novel cellular-based assay for the diagnosis of active Lyme disease

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Objective: The presence of IgG/IgM antibodies directed against *Borrelia* antigens is used as standard diagnosis for Lyme disease, but diagnosis based solely on these serological assays is often difficult. In the present study, the potential value of a cellular-based assay (SpiroFind) for the diagnosis of active Lyme disease was evaluated. The SpiroFind test is based on the capacity of peripheral blood mononuclear cells (PBMCs) from patients to build up a cellular-based immunological memory and produce enhanced cytokine levels upon re-stimulation with intact *Borrelia* species.

Methods: Patients suspected of Lyme disease were seen at the outpatient clinic for Lyme disease from the Department of Medicine, Radboud University Nijmegen Medical Centre. The diagnosis of Lyme disease was evaluated using standard *Borrelia* serology (Enzyme-linked immunosorbent assay or Western blot) or SpiroFind assay.

Results: The overall prevalence of *Borrelia* exposure in the tested individuals was similar in SpiroFind compared to both serological tests. The specificity of the SpiroFind was found to be comparable with the current serological tests. The similarity between serology and SpiroFind was 69%. However, the percentages of false-positive or false-negative outcomes using the SpiroFind were markedly lower as compared to serology.

Conclusion: SpiroFind revealed similar specificity for diagnosis of Lyme disease when compared to serology. Compared to serological tests, false-positive or false-negative results were found less often when using SpiroFind. Therefore, SpiroFind can be considered as alternative test next to the standard *Borrelia* IgG and IgM serology assays.

B037 (D035)

Development of a novel nucleic acid-based diagnostic assay for Lyme disease

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The infection with *Borrelia burgdorferi* can result in acute to chronic Lyme disease. The serological tests currently employed for its diagnosis are not very sensitive and are often difficult to interpret in a uniform manner by different laboratories. In addition, antibodies to *B. burgdorferi* persist long after the spirochete clearance, making it difficult to ascertain the cure of the disease after completion of the treatment regime. In addition, variation in the adaptive immunological response to different strains may affect the sensitivity. Finally, the test cannot be used for early disease before the adaptive immune response is induced. Therefore, there is a desperate need for a technically simple, rapid, and accurate assay to unequivocally diagnose active Lyme disease. Since nucleic acids of the pathogens usually do not persist after cure of the disease, DNA-based diagnostic tests are becoming highly useful for various infectious diseases. However, currently available PCR assays for Lyme disease are not very sensitive. Previously, we developed a quantitative real-time polymerase chain reaction (rt-PCR) technique using state of the art molecular beacon probes for detection of Lyme spirochetes presence in the infected mice tissues. Recently, we were able to successfully expand this strategy to detect the presence of *B. burgdorferi* even when they were present in very low numbers in the human DNA. Interestingly, this rt-PCR technique is also able to differentiate the simultaneous presence of all three major Lyme spirochete species, *B. burgdorferi, Borrelia afzelii*, and *Borrelia garinii* when present together by utilizing a post-PCR denaturation profile analysis, during which one molecular beacon probe distinguishes all the three species. This could be very useful for diagnosis and discrimination of various Lyme spirochetes in the European countries where all three species are prevalent. This novel diagnostic method will be able to use a variety of patient samples including blood, biopsy samples, cerebrospinal fluid, and synovial fluid for diagnosis of Lyme disease. We anticipate that pre-enrichment of the spirochetes present in the blood by culture before conducting the rt-PCR will further improve the sensitivity and specificity of the assay such that it can be used as a diagnosis of early to chronic stages of infection by Lyme spirochetes in the patients.

B038 (D038)

Performance of United States' serologic assays in the diagnosis of Lyme borreliosis acquired in Europe

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Background: American physicians sometimes need to evaluate a patient for suspected Lyme borreliosis (LB) who may have acquired the infection in Europe. Using serum samples from European LB patients, we compared the performance of European and U.S. serodiagnostic tests, including newer-generation assays containing VlsE or its C6 peptide.

Methods: The sensitivity of each assay was determined using 64 serum samples from LB patients with early or late disease manifestations who acquired the infection in Europe. Specificity was measured using 100 sera from healthy subjects from a non-endemic area.

Results: For the detection of European-acquired infection, conventional two-tiered testing (ELISA followed by immunoblotting) using U.S. assays had an overall sensitivity and specificity of 52 and 100% compared with 81% ($P = 0.0007$) and 99% ($P = 1.0$) using analogous European tests. The sensitivity of a U.S. C6 ELISA used as a stand-alone test (88% overall) was statistically comparable to that of conventional two-tiered testing using European tests ($P = 0.47$) and was 100% specific. Similarly, an alternative two-tiered algorithm using a standard U.S. ELISA followed by a C6 ELISA was comparably sensitive (84% overall) compared with conventional two-tiered testing using European assays ($P = 0.82$), and specificity remained 100%.

Conclusion: European assays outperformed analogous U.S. assays in a conventional two-tiered testing algorithm. However, a C6 ELISA used as a stand-alone test or in the second-tier of a two-tiered algorithm performed comparably to conventional two-tiered testing using European assays, and can be used for evaluation of any patient, regardless of travel history.

B039 (D042)

Analytical sensitivity of different PCR amplification protocols for detection of *Borrelia burgdorferi sensu lato*

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Background: Molecular detection and characterization of *Borrelia burgdorferi s.l.* (Bbsl) is important and widely used for research and diagnostics purposes. These methods enabled major progresses in virtually all fields regarding Lyme borreliosis, like diagnostic, pathogenesis, epidemiology, or prevention. However, one should be aware that standardization of the whole procedure and evaluation of the numerous methods are poor so far, which is especially crucial for diagnostic purposes. This pilot study aimed at testing the sensitivity of different Bbsl-DNA amplification protocols using identical DNA-material of a broad variety of Bbsl strains.

Materials and Methods: Sixteen different Bbsl strains – including all known human pathogenic species – *Treponema phagedenis*, *Borrelia hermsii*, *Leptospira biflexa* – Aliquots (n = 30 a 50 ml/each dilution step) of serial diluted DNA extractions [10,000-0.1 genome equivalents/PCR reaction (5 ml)] were frozen at 80°C – five different PCR protocols targeting OspA (n = 2), p41 (n = 2), or Hbb were included.

Results and Conclusion: Detection limit for a single PCR may vary from 1 to >10,000 genome equivalents/PCR reaction when testing different strains – detection limit for a single strain may vary from 1 to >10,000 genome equivalents/PCR reaction when using different amplification protocols – none of the protocols showed optimal amplification of all strains tested; therefore, none is suited as stand-alone test for diagnostic purposes There is urgent need for improvement and standardization of Bbsl PCR protocols, e.g., studies that identify well-suited PCR protocols and studies to further improve such protocols.

B040 (D043)

Xenodiagnosis using *Ixodes scapularis* larva in humans

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Background: *Borrelia burgdorferi*, the causative agent of Lyme disease, has been shown to persist in infected animals after antibiotic therapy. Whether *B. burgdorferi* persists in humans with Lyme disease after antibiotic therapy is unknown. One method for successfully detecting *Borrelia* in animals after antibiotic therapy is xenodiagnosis, which involves feeding uninfected ticks on animals in order to draw bacteria into the tick. Because this is part of the natural lifecycle, *B. burgdorferi* may be particularly adapted to recognizing the presence of a feeding tick and re-entering that tick. Xenodiagnosis using *Ixodes scapularis* ticks has not been studied in humans.

Methods: We examined the safety of xenodiagnosis in humans. Laboratory-reared *I. scapularis* larval ticks were placed on 33 subjects and allowed to feed to repletion. Ticks and skin biopsies were tested for the presence of *B. burgdorferi* by PCR, culture, fluorescent microscopy, and mass spectroscopy. Xenodiagnosis was repeated in seven individuals.

Results: Xenodiagnosis was well tolerated with no severe adverse events. The most common adverse event was mild itching at the site. Data from the testing of xenodiagnostic ticks are currently being analyzed, and we anticipate presenting these results at the meeting.

Conclusion: Xenodiagnosis is a safe method for recovering *B. burgdorferi* from subjects after treatment for Lyme disease. Further studies will be needed to determine the sensitivity of xenodiagnosis and whether the presence of organisms after antibiotic therapy is associated with symptoms in patients after treatment for Lyme disease.

B041 (D044)

Development of loop-mediated isothermal amplification (lamp) for relapsing fever

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Patients with relapsing fever (RF) are often misdiagnosed with malaria in areas where the diseases coexist. In an effort to address this issue, we have developed a novel tool for detection of RF using loop-mediated isothermal amplification (LAMP) for diagnosis in a basic healthcare setting. In LAMP, amplification and detection of a target gene can be completed under isothermal conditions in a single step. Two assays were designed, one for the North American pathogen *Borrelia hermsii* and the other for the African pathogens *Borrelia duttonii*, *Borrelia recurrentis*, and *Borrelia crocidurae*. Primers targeted the glycerophosphodiester phosphodiesterase (glpQ) gene. Primer specificity was evaluated in three ways: (i) restriction enzyme digestion of LAMP product, (ii) PCR with LAMP primers, and (iii) by running the assays with other *Borrelia* species. Based on these results, the assays were determined to be specific. The sensitivity of the assays was evaluated using qPCR. The African RF assay was highly sensitive, having a detection limit of six gene copies. The *Borrelia hermsii* assay, the North American pathogen, had a detection limit of 400 gene copies. We used a crude DNA extraction method to extract *Borrelia* DNA from whole blood. The LAMP assay was not inhibited by blood products in the crude DNA extract. During method development, issues with amplification products contamination arose. Further development to reduce the risk of amplification products contamination is necessary. Clinical sensitivity and specificity are under investigation using blood samples from patients in Rwanda. LAMP is a simple and reliable method for detecting RF and has the potential to improve diagnosis of RF in basic healthcare settings.

B042 (D046)

Autoantibodies to human endothelial cell growth factor in American and European patients with various manifestations of Lyme disease

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Background: In a search for autoantigens in antibiotic-refractory Lyme arthritis, we recently identified endothelial cell growth factor (ECGF) as a target of T and B cell responses in patients with erythema migrans (EM) or Lyme arthritis. Moreover, ECGF, an IFN-inducible protein, was expressed at significantly higher levels in synovial fluid of antibiotic-refractory versus antibiotic-responsive patients, and is also upregulated in neuronal cells under inflammatory conditions. Here, we tested for anti-ECGF autoantibodies in serum samples from American and European (Slovenian) patients with a range of Lyme disease manifestations.

Methods: Anti-ECGF antibodies were sought in serum samples from 344 American and 58 Slovenian patients with EM, Lyme neuroborreliosis (LNB), acrodermatitis (ACA), or responsive or refractory arthritis. Additionally, sera were tested from 27 non-antibiotic-treated American patients seen in the 1970s, who were followed longitudinally from early through late infection. A positive response was defined as >3 SD above the mean value in 74 healthy control subjects.

Results: Among antibiotic-treated American patients, 15% with EM, 22% with LNB, 8% with responsive arthritis, and 17% with refractory arthritis had positive ECGF antibody responses. Among patients with EM, symptoms resolved more slowly after treatment in those with ECGF reactivity (7 versus 3 days, $P = 0.01$), and ECGF responses were more common in patients with refractory versus responsive arthritis ($P = 0.1$). Of the 27 non-antibiotic-treated American patients followed from EM through the course of arthritis, 7 (26%) had ECGF antibody responses, which often appeared early in the illness. Moreover, during early disseminated infection, 4 of the 7 ECGF-positive patients (57%) developed meningoencephalitis compared with 2 of the 20 anti-ECGF antibody-negative patients (10%) ($P = 0.02$). During the period of persistent infection, the duration of active arthritis was significantly longer in those with ECGF reactivity (67 versus 17 weeks, $P = 0.004$). In contrast, a smaller percentage of Slovenian patients had ECGF antibody responses (7–9% with EM, LNB, or arthritis), except for those with ACA in whom 38% had ECGF reactivity.

Conclusion: American and European patients with various manifestations of Lyme disease had ECGF antibody responses, but their frequencies tended to be greater in Americans. We postulate that patients who experience more severe and prolonged inflammation (Americans with meningoencephalitis or refractory arthritis or Europeans with ACA) have higher IFN levels and greater ECGF expression. This may lead to enhanced presentation of ECGF self-epitopes and autoantibody production. In individuals susceptible to immune dysregulation, these auto-antibodies may contribute to persistent inflammation and more severe and prolonged disease.

B043 (D047)

Ixodes tick-borne borreliosis with antibody response to glpQ antigen of *Borrelia miyamotoi*

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Human cases of *Ixodes* tick-borne borrelioses (ITBB) in Russia may be caused either by *Borrelia burgdorferi* s.l. or by *Borrelia miyamotoi*. These infections may be detected by amplification of *Borrelia* DNA in blood using specific PCR. The primary acute clinical manifestation of *B. burgdorferi* infection is the erythema migrans (EM) rash, whereas *B. miyamotoi* infection usually presents as a flu-like disease with high fever but without EM. Serological methods can support and supplement PCR-based diagnostics. Unfortunately, commercial ELISA and Western blot assays using *B. burgdorferi* antigens cross-react with some antigens of *B. miyamotoi*, so they are not useful in discriminating between these two diseases. *B. miyamotoi* is part of the relapsing fever group of *Borrelia* species that produce glpQ protein, which is absent in *B. burgdorferi* s.l. species. We have developed an immunofluorescence microarray that includes eight recombinant antigens (p100, VlsE, p58, p41, p39, BBK32, OspC, and p17) of *Borrelia afzelii* and *Borrelia garinii* from Russian tick isolates, and a *B. miyamotoi* glpQ recombinant antigen from blood of patient in the Ural Region of Russia. We tested this array on serum samples collected from ITBB patients living in Udmurtia in 2012. The first group included nine patients without EM who had fever, headache, and fatigue. *B. miyamotoi* DNA was amplified from admission blood samples using *B. miyamotoi*-specific PCR. Anti-glpQ IgM antibody was found in six of the nine serum samples collected 2 days after disease onset, all the nine serum samples collected at 12 days after disease onset, and all the six serum samples collected 35 days after a disease onset. Anti-glpQ IgG antibody was found in one of the nine samples, three of the nine samples, and five of the six samples, respectively. A second group of study subjects consisted of 12 patients with EM. *B. miyamotoi* DNA was not detected in their blood. The level of anti-glpQ IgM and IgG antibodies was below threshold in all serum samples taken from this group of patients at days 6 and 15 after disease onset, with the exception of one IgM positive sample and another IgG positive sample. The pattern of response to eight non-glpQ antigens was more diverse. The patients from both groups produced IgM antibody to at least two of the four antigens (VlsE, OspC, p41, and p17) and IgG antibody to at least two of the eight non-glpQ antigens. Patients with PCR evidence of *B. miyamotoi* infection have detectable anti-glpQ IgM and IgG antibodies in their acute and convalescent sera while patients with *B. burgdorferi* s.l. do not.

B044 (D048)

Unusual clinical presentations of *acrodermatitis chronica atrophicans*

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Background: Acrodermatitis chronica atrophicans (ACA) is a late cutaneous manifestation of Lyme disease defined as erythematous to livid discoloration and skin atrophy, mostly at the extensor surfaces of the legs and rarely in the face, initially with oedematous swelling, occurring months to years after infection with *Borrelia burgdorferi.* High IgG antibody titres are typically found. We report the cases of two ACA patients with unusual clinical and laboratory presentations. Patient 1: an 80-year-old patient showed diffuse large erythema with teleangiecatasias on the trunk extending on the back, the legs showed no such lesions. The patient recalled a tick bite 5 years earlier, but had no history of erythema migrans. Laboratory results revealed highly positive *Borrelia*-specific antibody titres for both IgM and IgG. Histology showed lymphoplasmocytic infiltrates without fibrotic changes. PCR from histology was positive for *B. burgdorferi*-DNA. The patient was treated with doxycycline 200 mg for 30 days, and the lesions resolved almost completely within 6 months. Patient 2: a 76-year-old woman presented to her dermatologist with a livid plaque on the right ankle, 7 cm in size. The patient did not recall a tick bite or an erythema migrans. Histology was compatible with ACA; however, serology showed positive IgM antibodies by ELISA, but was negative for IgG antibodies. Immunoblot diagnosis was negative. PCR from histology was carried out and revealed *B. burgdorferi*-DNA. Treatment with doxycycline for 30 days leads to complete resolution of the plaque. At the end of the treatment, IgG antibodies became positive by ELISA; however, confirmatory immunoblot remained negative.

Conclusion: ACA typically occurs at the extremities, while morphea is also found on the trunk. However, ACA may also occur in regions other than the distal extremities, although only a few cases have been reported in the literature so far. Seronegative ACAs are a very rare finding and might be due to variety of borrelial strains or might be associated with HLA-specificity.

B045 (D049)

Development of a new multiplex early diagnostic assay for Lyme borreliosis based on antigens expressed in the skin of the mammalian host

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Current immunological assays used to diagnose exposure to *Borrelia burgdorferi* suffer from low sensitivity during the early stages of infection. Our hypothesis is that we could improve early detection of Lyme borreliosis by detecting antibodies recognizing antigens expressed by spirochetes as they infect and begin to disseminate within the mammalian host. Toward this end, we used whole *B. burgdorferi* genome microarrays to identify genes expressed by *B. burgdorferi* isolated from the skin of MyD88$^{-/-}$ mice infected with *B. burgdorferi* strain 297. These immunodeficient mice mount an antibody response against the bacterium that appears identical on immunoblot to wild-type mice, but the pathogen can be found in higher numbers in the skin and other tissues after infection. We selected 11 proteins for initial study based on a variety of criteria: (1) conservation among strains of *Borrelia*; (2) lack of homology to proteins from unrelated organisms; (3) presence of a lipobox; and (4) higher level of expression in the skin compared to liquid culture in BSK-II medium. These proteins were expressed in recombinant form in *Escherichia coli* and coupled to Luminex beads, along with recombinant OspC from *B. burgdorferi* strains 297 and B31. The prototype Luminex assay containing 12 different antigens detected IgG and IgM antibodies in a cohort of 200 human sera that had previously tested positive using a C6 ELISA + immunoblot assay, proving that the selected proteins were expressed and immunogenic in infected human patients. Antibodies against individual antigens were detected in 25–89% of samples. More than 85% of patient serum samples were positive for at least three antigens while serum samples from patients with no history of Lyme borreliosis were positive for 0–2 antigens. We also analyzed serum samples from 15 rheumatoid arthritis patients with no history of Lyme disease. With one exception, all patients tested negative. We next analyzed 134 serum samples collected during a serosurvey in Block Island, RI, in 2012. Thirty-three of 134 samples were IgG-positive in our prototype multiplex assay, and 17 of those were confirmed positive by immunoblotting. Results of C6 ELISA (Immunetics) and immunoblot analysis (Viralab) on the same specimens are pending. Over the next several months, we will be conducting a prospective study of sera from patients presenting with erythema migrans to compare the sensitivity and specificity of our assay with that of existing commercial assays for the detection of early Lyme disease.

B046 (D050)

Diagnosis of Lyme neuroborreliosis by combination of antigen-specific antibody index with CXCL13 cytokine determination

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Lyme neuroborreliosis (LNB) is a nervous system infection caused by *Borrelia burgdorferi sensu lato* (Bb). The diagnosis of defined LNB is supported by: (i) neurological symptoms, (ii) a lymphocytic pleocytosis in the cerebrospinal fluid (CSF) and (iii) intrathecally produced *Borrelia burgdorferi* (Bb)-specific antibodies. Bb-specific antibodies in serum and CSF can be detected with IgG- and IgM-differentiating assays but antibody determination has to deal with low antibody titers in the very early phase of the disease and delayed antibody titer decrease following therapy. Recent studies have suggested that the B-cell attracting cytokine CXCL13 is reliably increased in the CSF of patients with well-defined early LNB, thus determination of CXCL13 might be helpful in seronegative patients during early disease and for control of therapy. We present a rapid and convenient particle immunoassay (PIA) combining the calculation of an elevated antibody index (AI) with the quantitative detection of CXCL13. On the basis of the xMAP-Technology, a serological test-system for diagnosis of Lyme borreliosis including CSF/serum antibody index (AI) calculation was developed. In this assay, 13 recombinant, immunodominant antigens (p100, VlsE, p58, p39, OspA, OspC, p18) of the five pathogenic genospecies: *B. burgdorferi sensu strictu*, *Borrelia garinii, Borrelia afzelii*, *Borrelia spielmanii* and *Borrelia bavariensis*, were immobilized to a single color coded population of polystyrene beads each. In addition, antibodies against the cytokine CXCL13 were immobilized to a 14th bead population. CSF/serum antibody index (AI) and CXCL13 concentration were determined in three different patient panels: 30 samples with normal AI, 148 routine samples with suspected neuroborreliosis and 38 samples from clinically well-defined early neuroborreliosis. Each single antibody/antigen reaction could be measured quantitatively with high reproducibility. The linear range covers three log levels. The lower detection limit of CXCL13 was 50 pg/ml and the system showed a linear range up to 10,000 pg/ml. We compared the CXCL13 concentrations in all the three groups for significant correlation. The evaluation of the selected patient panels show an increasing number of CXCL13 positives depending on the stage of infection. The combination of antigen-specific antibody index calculation with CXCL13 cytokine determination is a promising tool for the diagnosis of Lyme neuroborreliosis.

B047 (D053)

PCR-based diagnostics of Lyme disease using capillary blood obtained from erythema migrans lesion

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Introduction: Routine laboratory diagnosis of Lyme disease (LD) is based on the detection of specific *Borrelia burgdorferi* antibodies by ELISA, Western blot, or microarrays. Antibody testing is of limited value because anti-borrelial IgM (and particularly IgG) usually only appears after several weeks after the onset of infection or may not be produced at all. PCR-based assays are an option for early detection of *B. burgdorferi* infection; however, in the absence of systemic infection, *B. burgdorferi* DNA is usually not found in venous blood. In our laboratory, only 15–30% of blood samples taken on admission from patients with confirmed LD are PCR positive. The proportion of positive PCR results may increase to 40–80% if skin biopsy specimens are used. The skin biopsy procedure may be traumatic and is not recommended for LD diagnosis in Russia. We have therefore developed and tested a novel approach using capillary blood sample (CBS) that are obtained from erythema migrans (EM) lesions.

Methods: Twenty patients with LD living in Izhevsk City, Russia, in 2012 were enrolled in the study. First sampling was done at admission, from 2 to 20 days after the appearance of a typical EM (³5 cm in diameter) and before the start of antibiotic treatment. Venous blood was taken for standard ELISA and PCR testing. Simultaneously, 0.1 ml of CBS was taken from the EM lesion, supplemented by EDTA, and subjected to DNA extraction and PCR. Commercial PCR test-system "AmpliSens® TBEV, *B. burgdorferi* s.l., *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis/Ehrlichia muris-FL*" was used according to manufacturer's instructions (www.interlabservise.ru).

Results: Of 20 patients with LD, *B. burgdorferi* DNA was amplified from the venous blood of four patients using specific real-time PCR, whereas three of these patients tested positive using the CBS assay and one tested negative. Six additional PCR-positive results were obtained using the CBS assay. Seroconversion for specific IgM and/or IgG was noted in 14 of the 20 serum samples taken about 10 days after the first sampling, including 8 of 10 samples from PCR-positive patients. The proportion of PCR-positive results in our CBS assay (9 of 20 samples) was similar to that when we tested 2 mm skin biopsy specimens in 2010–2011 (9 of 17 patients) using PCR.

Conclusion: CBS is an effective non-traumatic method for early detection of the *B. burgdorferi* pathogen. It may be particularly useful if the EM is <5 cm in diameter or has an ambiguous appearance.

B048 (D055)

Direct molecular detection of *Borrelia burgdorferi* from whole blood and CSF of patients with acute and chronic Lyme disease (revised)

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The early detection and treatment of Lyme disease is crucial to prevent late sequelae and to improve long-term prognosis. However, infection is often difficult to diagnose because of the variability of clinical manifestations and the biologically delayed antibody production upon which current serologic tests are based. Furthermore, serological tests cannot be used to show response to treatment as a patient can remain sero-positive for years following exposure. Direct molecular tests have the ability to detect *Borrelia* both before and after patients seroconvert, thus aiding in the earlier diagnosis and subsequent assessment of the response to treatment. However, one of the challenges of direct molecular tests is that even during acute infection, there are relatively few *Borrelia* present in the blood of the patient. To address this challenge, we have developed an "ultra-sensitive" molecular assay to detect *B. burgdorferi* directly from whole blood, CSF, or other specimens collected as early as the initial patient visit. This molecular assay can detect less than a single genome of *Borrelia* DNA in a specimen. Furthermore, this approach also has the potential to detect *Borrelia* in patients who have already been treated for Lyme disease. To achieve increased sensitivity, we employed a larger than typical sample volume (1.25 vs. 0.2 mL). Secondly, we performed an isothermal pre-amplification of the *Borrelia* DNA (akin to a "molecular culture") to increase the copies of *Borrelia* DNA in the sample above the limits of detection of PCR. Thirdly, we detected the enriched *Borrelia* DNA with a broad-range PCR and electro-spray ionization mass spectrometry assay that targets eight *Borrelia* loci each of which is diagnostic for *Borrelia*. This assay not only identifies the presence of *Borrelia* DNA but can also indentify the infecting *Borrelia* species and frequently its genotype. To test this technology, we evaluated the assay on blood that had been collected at the initial presentation of 21 endemic-area patients who had both physician-diagnosed erythema migrans (EM) and positive two-tiered serology either at the initial visit or at a follow-up visit after 3 weeks of antibiotic therapy. The results of this study demonstrated the ability to detect *B. burgdorferi* in 13 of the 21 patients (62%). In 5 of the 21 cases, we demonstrated the ability to detect *B. burgdorferi* prior to seroconversion. Results will also be presented on the analysis of a large set (>200) of CSF specimens collected in Germany from patients with acute and chronic neuroborreosis.

B049 (D056)

The C6 assay – a Scottish perspective

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The C6 Lyme ELISA has generated a great deal of interest throughout Europe and the USA, where it has been proposed as a sensitive screening assay, an alternative to two-tier testing, and a marker of treatment response or resolving infection. The aim of this pilot study was to assess these roles of the C6 assay in a routine diagnostic laboratory. Sera (n = 252) from patients with clinically suspected Lyme borreliosis were tested by both the C6 Lyme ELISA (Immunetics) and our current screening test, the Enzygnost Lyme link VlsE/IgG ELISA (Siemens), as per the manufacturer's instructions. Reactive samples were confirmed by commercial EU Lyme IgG Western blot (Trinity biotech) and/or in-house IgG Western blot. Overall, the Enzygnost assay was more sensitive than the C6 ELISA (182/252 vs. 168/252 reactive sera, respectively). Since the C6 assay is less sensitive and more expensive, it would not replace the current screening test. Detection of positive sera with single-tiered testing with both EIAs was equivalent to two-tiered testing with in-house IgG Western blot confirmation of equivocal/positive screening sera but was more sensitive than two-tiered testing with Trinity IgG Western blot confirmation. The decrease in sensitivity with the two-tiered testing utilizing the Trinity Western blot (85/152 vs. 55/152 and 79/152 vs. 62/152 positive results with the C6 and Enzygnost assays, respectively) may be due in part to the sensitivity of this particular assay, or a reflection of superior specificity. Analysis of the clinical information from these patients will determine the performance of the single- and two-tiered testing protocols. Paired sera from 18 patients were tested in parallel with the C6 and Enzygnost assays and either the Trinity or in-house IgG Western blots to monitor treatment response or indicate resolving infection. There was no difference in paired sera results for 11 patients. In five patients, there were quantitative differences in the both the C6 and Enzygnost assays and Western blot results. One patient had a quantitative decrease with the Enzygnost ELISA and Western blot result between subsequent samples but no difference with the C6 ELISA. A further patient had a quantitative increase with both the C6 and Enzygnost assays in subsequent samples but no difference in Western blot pattern. Although the numbers of samples in this study is small, the early indication suggests that there is no additional advantage to utilizing the C6 assay as a tool for monitoring treatment response or resolving infection.

B051 (P003)

Antigenic analysis of North American *Borrelia burgdorferi sensu lato* isolates

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A large number of spirochete species belonging to *Borrelia burgdorferi sensu lato* (s.l.) complex have been identified worldwide. At least eight different species have been isolated in the United States and to date only *B. burgdorferi sensu stricto* (s.s.) is believed to cause human Lyme borreliosis. In this study, antigenic analysis was conducted for six of the US *B. burgdorferi* s.l. species. Mice were inoculated with *Borrelia americana, Borrelia andersonii*, *Borrelia bissettii, Borrelia carolinensis*, *Borrelia kurtenbachii* and *B. burgdorferi* s.s. Immunoblots of *Borrelia* exposed mouse sera against each of the *Borrelia* whole cell lysates revealed multiple common immunogenic proteins, yet also revealed immunogenic proteins specific to *Borrelia* exposure history. Western blots to specific proteins will allow us to determine the specific response to the more common immunogenic proteins, such as VlsE, OspA, OspC, Flagellin, DpbA, DpbB, and BBK32. *B. bissettii* also stimulated an antibody response to the diagnostic C6 peptide as strong as *B. burgdorferi* s.s. These preliminary results indicate that *B. bissettii* expresses VlsE variable antigen and that the C6 peptide ELISA would remain to be an effective serological tool if 1 day *B. bissettii* is confirmed to cause human disease. And because most of these *Borrelia* have yet to be associated with human disease, characterization of the immune response to these spirochetes may help identify *Borrelia*-specific immunogenic proteins.

B052

Enzyme-linked immunosorbent assay to detect anti-Lyme IgM and IgG antibodies in sera of patients resident in Kuwait

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Lyme disease caused by *Borrelia burgdorferi* is uncommon in Kuwait, but people visiting endemic countries may get infected and develop symptoms when they come back to Kuwait. The aim of this study was to determine the serological evidence of infection in people who returned back from endemic countries and were clinically suspected of Lyme disease. A total of 80 sera, obtained from patients attending five major hospitals in Kuwait, during 2009–2013, were tested for anti-Lyme IgM and IgG antibodies using enzyme-linked immunosorbent assay (ELISA). The samples were tested using a commercial assay (Serion ELISA Classic, Germany). The test employs a positive control, a negative control, and a cut off serum. To achieve a high level of sensitivity, a combination of a European strain of the genus *Borrelia garini* along with *Borrelia afzelli* PKo was used. The antigen cocktail had the antigen OspC and VisE, which are particularly important for the diagnosis of early stage *Borrelia* disease, in addition to the late stage critical protein P100. In order to further increase the sensitivity, a recombinant VisE was added to the coating antigen complex. Specificity was optimized by the addition of a lysate of *Treponema phagedenis* to the dilution buffer which selectively absorbs any cross reacting *Treponema* antibodies, which may be present. The results showed that 32/80 (40%) sera were antibody positive. Out of these, 20 were positive for IgM only, 6 for IgG only and 6 for both IgM and IgG. The results suggest that ELISA could be useful for serological evidence of infection with *B. burgdorferi* due to its immunoglobulin specific analysis of the immune response. The study was supported by Kuwait University Research Sector grant GM01/01.

B053

From prevalence studies to the development of novel diagnostic tests for Lyme disease

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Worldwide, tick-borne diseases seem to become increasingly important for public health, of which Lyme borreliosis is of main importance for Europe. Strategies to assess the risk for contracting tick-borne diseases are, e.g., pathogen surveillance in ticks and determination of seroprevalence in risk groups. Here, we analyzed 5638 ticks that were collected in Luxembourg between 2007 and 2009 and screened blood samples from more than 250 forestry workers collected in the years 2010, 2012, and 2013. Pathogen-specific detection PCRs revealed that 16.3% of questing ticks were infected with *Borrelia burgdorferi* sensu lato, the causative agent of Lyme borreliosis. Other tick-borne pathogens were less prevalent, e.g., Spotted fever group *Rickettsiae* (6.7%), *Babesia* species (1.8%), *Anaplasma phagocytophilum* (0.9%), and *Bartonella* species (0.1%). Tick-borne encephalitis virus *Coxiella burnetii* and *Francisella tularensis* were not found in ticks from Luxembourg. Seroprevalence rates of *B. burgdorferi* s.l. in forestry workers were on average 31% for IgG and 13% for IgM antibodies. Only a proportion of the seropositive forestry workers reported to have been previously diagnosed for Lyme borreliosis, which is in line with reports of several infections with borrelial spirochetes remaining asymptomatic. Within the period from 2010 to 2012, seroconversions were observed for about 1.7% for IgG and 8.7% for IgM. None of the IgM positive candidates analyzed in 2010 (n = 14) converted back to negative within the 2 years period of screening. In contrast, 8.1% of the IgG initially positive individuals were negative in 2012. In an aged and sex matched control group from 2011, 4% displayed IgG and 3% IgM antibodies against *B. burgdorferi* s.l.. These data suggest that Lyme borreliosis is an important health concern in Luxembourg, especially among professional high risk groups. As the diagnosis of Lyme borreliosis is complicated by the often unspecific symptoms and the absence of a reliable diagnostic test of the active disease, our institute currently investigates strategies to shed light on the complex immune response elicited by the pathogen, thus contributing to the development of novel diagnostic tests. So far, the response of the adaptive immune system to *Borrelia* infection is not completely understood. Therefore, antigen-specific B-cells will be isolated and compared on a single cell level between acute, chronic, and resolved patients. With this approach, we expect to identify *Borrelia* specific B-cell clones, which may be valuable tools for epidemiology, symptomatology, and perhaps even diagnosis.

B054 (Ep005)

Emergence of babesiosis in Maine: entomological correlates of human seroprevalence and disease

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Babesia microti was first documented in *Ixodes scapularis* ticks in Maine in 1996, and the first human case was reported in 2001. Despite presence of vector ticks in all Maine counties, and reports of 900–1000 cases of Lyme disease per year, human babesiosis emerged relatively recently with more geographic focality. We sought to determine whether human cases correlated with *I. scapularis* abundance and tick infection prevalence of *Babesia microti* and *Borrelia burgdorferi*.

Methods: We reviewed cases of human babesiosis reported to the Maine CDC from 2001 to present and complete dentomologic surveys at several established field sites from 1995 to 2012 to determine abundance of *I. scapularis* ticks and infection prevalence by *B. burgdoferi* and/or *B. microti*. In addition, we completed a serosurvey for antibody positivity to *Babesia* in healthy blood donors from an area highly endemic for *B. burgdorferi*-infected ticks. Infection by *B. burgdorferi* was detected by direct fluorescent antibody and/or PCR directed against *ospC*. *Babesia* was detected by PCR and sequenced to distinguish *B. microti* from *Babesia odocoilei*. Antibody (FITC fluorescent antibabesial specific IgG) was detected in serum samples from human serosurvey participants by IFA examination at a dilution of 1:64.

Results: Reported human cases increased from 0–5 cases/years in 2000–2004 to 5–11 cases/years in 2005–2011. Cases were marginally correlated with *I. scapularis* tick abundance (Spearman correlation coefficient = 0.61, $P = 0.11$), and *B. microti* infection percent (0.66, $P = 0.07$), and correlated with *B. burgdorferi* infection percent (0.91, $P = 0.007$). Also, *B. microti* infection percent was correlated with abundance of *I. scapularis* ticks (0.73, $P = 0.04$). In one study site, we documented a shift in presence of *Babesia* spp. from *B. odocoilei* to *B. microti* between 1996 and 2011. Seroprevalence of anti-*Babesia* antibodies was 3.2% in healthy blood donors ($n = 311$), but no active infections were identified by PCR.

Conclusion: Human babesiosis was recently recognized in Maine 5 years after first detection in vector ticks, and 15 years after recognition of Lyme disease in the state. Prevalence of *B. microti* infection in ticks is correlated with abundance of *I. scapularis* and with prevalence of tick infections by *B. burgdorferi*. Our data are consistent with the hypothesis that human babesiosis may occur after attainment of higher tick densities than are associated with emergence of Lyme disease, possibly due to differences in mechanisms of pathogen dispersal and in enzootic transmission dynamics.

B055 (Ep007)

Self-reported Lyme disease diagnosis, treatment, and recovery: results from 2009, 2011, and 2012 HealthStyles nationwide surveys

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Background: Lyme disease (LD) is the most common vector-borne disease in the U.S. and the sixth most common nationally notifiable disease. The Centers for Disease Control and Prevention (CDC) has a number of projects underway to evaluate key measures of LD burden in the U.S. As part of this overall effort, we utilized several nationwide HealthStyles surveys to investigate patterns of LD diagnosis, treatment, and recovery.

Methods: HealthStyles surveys are annual, cross-sectional surveys containing questions on knowledge, attitudes, and behaviors for health-related topics. These surveys, conducted by mail or online, are designed to be demographically representative of the U.S. population, and samples are weighted based on census regions and several demographic variables. We included a total of seven questions related to self-reported LD diagnosis, treatment received, recovery time, and persistence of symptoms in the 2009, 2011, and 2012 HealthStyles surveys. Analysis of responses was primarily descriptive.

Results: Response rates were 73% (4728/6504) in 2009, 69% (4050/5868) in 2011, and 80% (3503/4371) in 2012. Overall, 51% of respondents were female, 71% were white, 65% had an annual household income ³$40,000, and 64% reported some college education. Median respondent age was 51 years. Fifty-nine (1.3%) respondents in 2009 and 32 (0.9%) in 2012 reported having ever been diagnosed with LD; 10 (0.3%) respondents in 2012 were diagnosed in the last year. Among 26 respondents in 2012 with a past diagnosis of LD (defined as diagnosis >6 months earlier), 15 (58%) reported recovering fully in <6 months, 8 (31%) recovered in 7 months to 2 years, and 3 (12%) remained ill for >3 years. The most common persistent symptoms experienced by 2012 respondents who had not recovered fully were fatigue, muscle aches, joint swelling, and numbness or tingling; none reported receiving intravenous antibiotics or other alternative treatments. In 2009, 23 (39%) of 56 respondents receiving antibiotics were treated <4 weeks, while 21 (36%) were treated >8 weeks. Overall, 426 (10.5%) respondents in 2011 reported personally knowing someone described as having “chronic LD.”

Conclusion: Our results are subject to several limitations, including self-reported diagnoses and wide confidence intervals associated with the outcomes of interest. Nevertheless, applied to a population of >300 million, our findings from these nationally representative surveys suggest that a very large number of individuals in the U.S. have been diagnosed with LD. Although most patients recovered in <6 months, a significant proportion received antibiotics for >8 weeks.

B056 (Ep008)

Investigation of tick-borne encephalitis virus in ticks detached from humans with follow-up of clinical symptoms and serological response

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Tick-borne encephalitis virus (TBEV) is a serious cause of acute central nervous system disease in large parts of Europe and Asia. In Sweden at present, 200–300 cases of tick-borne encephalitis (TBE) are registered each year, showing an increasing trend and a spread to new areas. In order to investigate the risk of acquiring TBE after a single tick-bite, the tick-borne diseases (TBD) STING study was set up to collect ticks, blood samples, and questionnaires from newly tick-bitten humans at participating primary health care centers in Sweden and at the Åland Islands, Finland. We received a total of 2167 ticks from 1886 newly tick-bitten individuals during 2008–2009. Using quantitative real-time PCR, we found five ticks infected with TBEV, with highly varying virus copy numbers. Three individuals from the Åland Islands bitten by TBEV-infected ticks were vaccinated. One person bitten by a tick carrying a high viral load was not vaccinated, and experienced a short episode of fever consistent with the viremic phase of European subtype TBEV infection. The other non-vaccinated person was bitten by a tick carrying few TBEV copies, but did neither develop any symptoms nor anti-TBEV antibodies. Two other persons developed new anti-TBEV antibodies during the 3 month study period, but TBEV could not be detected in the ticks from these two persons. One of these two persons reported headache, fatigue, neck pain, stiffness, dizziness, and concentration difficulties during the study time. In conclusion, very few ticks were infected with TBEV in the investigated regions in the TBD STING study, and the risk of acquiring TBE after a single tick-bite seems to be low.

B058 (Ep010)

The epidemiology of Lyme disease in Ontario, 2007–2011

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Background: Lyme disease is reportable in Ontario and provincial notification of human cases occurs through the integrated Public Health Information System (iPHIS). Complementary passive tick surveillance, whereby the public submits ticks to public health for identification and testing, and active tick surveillance, whereby public health authorities actively look for and test ticks, are also conducted in Ontario. This poster will describe the epidemiology of Lyme disease in both the human and blacklegged tick populations in Ontario.

Methods: Human Lyme disease cases were obtained from iPHIS and tick data were obtained from the tick submission database for the years 2007–2011. Using SPSS 18, changes in incidence over time were evaluated using Poisson regression and differences in proportions were tested using the chi-square statistic, with both assessed at the 95% level of significance.

Results: In Ontario, 515 human cases of Lyme disease were reported from 2007 to 2011 for an average of 103 cases per year. The annual incidence rate of Lyme disease increased steadily over the period from 0.60 cases per 100,000 population in 2007 to 1.01 cases per 100,000 population in 2011. Cases ranged in age from 2 to 84 years, with a mean age of 43.1 years; over half (56%) of the cases were female. The majority of cases reported an exposure, including 173 travel-related and 277 locally acquired cases. Of the locally acquired cases, 157 (57%) were associated with exposures in non-endemic areas in Ontario, 98 (35%) with exposures in established endemic areas, and 22 (8%) with unspecified exposures within Ontario. Blacklegged tick submissions to public health also increased from 1,171 submissions in 2007 to 2,286 in 2011.

Discussion: Since 2007, Ontario has experienced an increase in the incidence of human Lyme disease cases, as well as an increase in the number and geographic range of the blacklegged tick vector. This trend may be explained by greater public awareness that has been resulted in increased tick submissions. A simultaneous increase in the demand for Lyme disease testing also occurred over this period as evidenced by the 50% increase in the number of human specimens submitted to public health labs for diagnosis. In Ontario, the geographic distribution of human cases and blacklegged ticks show strong geographic correlation, indicating increased risk of human cases.

B059 (Ep011)

Low risk of seroconversion and clinical disease after bite by an *Anaplasma phagocytophilum*-infected tick

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Background: The tick-transmitted bacteria *Anaplasma phagocytophilum* is known to occasionally cause human granulocytic anaplasmosis (HGA). HGA is typically a febrile illness with headache, myalgia, and malaise. The symptoms are generally mild, and subclinical infections are common. Since *A. phagocytophilum* share vectors with *Borrelia burgdorferi* sensu lato (s.l.), co-infections occur. Data from animal studies suggest that such co-infections are more severe, possibly due to immunosuppression induced by *A. phagocytophilum*. However, the importance of *A. phagocytophilum* as a human pathogen is still uncertain, and little is known about co-infections with *B. burgdorferi* s.l., as well as the risk of contracting HGA after a tick-bite. The aim of this study was to evaluate the risk for humans of developing HGA or subclinical seroconversion after being bitten by an *A. phagocytophilum*-infected tick.

Methods: This study was part of the on-going Sting project, in which tick-bitten individuals are asked to donate the tick along with a blood sample. Three months later, the participants are asked to give a second blood sample. Participants are also asked to fill in questionnaires concerning symptoms, etc. 2154 ticks collected in 2008–2009 from Sweden and on the Åland Islands, Finland, were analyzed for *A. phagocytophilum* and *B. burgdorferi* s.l. DNA using real time PCR. Feeding time was estimated using the coxal and scutal indices. Paired serum samples from the tick-bitten individuals were analyzed for IgG-antibodies against *A. phagocytophilum* using indirect immunofluorescence.

Preliminary Results: Thirty (1.4%) ticks were positive for *A. phagocytophilum*, seven of these were co-infected with *B. burgdorferi* s.l., 556 (26%) ticks were positive for *B. burgdorferi* s.l. Anaplasma serology was performed on serum pairs from the 30 individuals bitten by *A. phagocytophilum*-infected ticks (group A), 30 individuals bitten by *B. burgdorferi* s.l.-infected ticks (group B), and 30 individuals bitten by ticks negative for both pathogens (group C). The overall seroprevalence in groups A–C was 18%, with no significant difference between groups. Seven individuals in group A reported symptoms (headache, neck pain, fatigue, vertigo), but none displayed significant increase in Anaplasma antibody titer or seroconversion. The estimated feeding time of the ticks in group A was 15–69 h (mean 33 h).

Conclusions: The risk of contracting HGA after a tick-bite is low, even if the tick is infected with *A. phagocytophilum* and has been feeding >24 h. The reported symptoms in group A is probably not related to HGA. The seroprevalence of 18% is in accordance with previous studies from Sweden.

B060 (Ep012)

Clustering of human Lyme disease by residential location in an endemic county, 2001–2011

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Background: Lyme disease (LD) incidence differs in space and time even within highly endemic states. Spatial dependence and clustering of human cases have not been quantified on a sub-county scale. To describe the clustering of LD cases and evaluate environmental and demographic risk factors for LD, we conducted a detailed analysis in a single, highly endemic county in Maryland.

Methods: We used human LD surveillance information from Howard County, Maryland, for 2001–2011. Residential address for all county households was available from the county Geographic Information Systems Division. Land use/land cover and census block group datasets were obtained from United States Geological Survey GAP Analysis Program, the State of Maryland, and ESRI Inc. Residence was assumed to be the exposure location. Spatial clusters were identified using SaTScan software, which takes into account the background population and calculates a relative risk (RR) based on observed versus expected cases. Spatial data were extracted and analyzed using ArcGIS and SAS. Initially, maximum cluster size was the software default of 50% of the population; subsequent analysis used a 2 km maximum cluster radius.

Results: Geocoded residence was available for 1684 (87%) of 1934 LD cases reported during 2001–2011. Using liberal cluster size criteria, elevated risk was evident for the entire western and central part of the county, an area with substantial agriculture and low density development (RR: 2.0, $p < 0.0001$). The eastern part of the county, with higher density development, displayed lower than expected risk (RR: 0.41, $p < 0.0001$). To identify more informative fine-scale risk factors, we restricted analyses to the western and central portion of the county. Six smaller areas of elevated risk (radius: 0.25–2 km) were identified (median RR: 3.17). Preliminary analyses indicate that when compared to other areas in this part of the county, these higher risk clusters had significantly more red and white-oak forest (median: 38 vs. 8%), regardless of amount of total forest. There were no differences in density of development or in census demographic factors. Soil type and forest fragmentation will also be examined.

Conclusions: We quantified the degree of spatial clustering of human LD risk within a single highly endemic county. High risk clusters were very similar to immediately surrounding areas except they contained a higher proportion of a single forest type. A better understanding of the factors driving the spatial distribution of human disease is needed to guide the development and implementation of effective prevention measures.

B061 (Ep013)

Lyme disease at a tertiary Lyme referral center in the Netherlands

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Introduction: Among patients and physicians, anxiety about Lyme disease (LD) leads to unnecessary diagnostic testing, antibiotic treatment, and misdiagnosis. In an attempt to provide better care to LD patients in the Netherlands, the Amsterdam Multidisciplinary Lyme Center (AMLC) was instituted consisting of various medical specialists (infectious diseases, neurology, dermatology, rheumatology, psychiatry and microbiology).

Methods: We retrospectively reviewed the first 100 adults referred to the AMLC. LD definitions were based on Stanek et al. (2011) and Wormser et al. (2006).

Results: Pretreatment characteristics were gender (56% female) and age (45.1 ± 13.9). The most frequently reported symptoms were fatigue (71%), arthralgia (47%), and tingling sensations (29%). Furthermore, 59% had symptoms lasting more than a year and 55% recollected tick bites. In 81% of patients *Borrelia* serology was performed prior to referral and 83 courses of antibiotics were prescribed (to 50% of the patients). At the AMLC, testing for *Borrelia* antibodies with C6 EIAs and/or immunoblots demonstrated a significantly higher C6-index in LD patients compared to non-LD patients. Of all patients, 49% were diagnosed with current LD – 6% EM, 4% early disseminated LD (two neuroborreliosis and two multiple EM), 11% late disseminated LD (two ACA, three Lyme arthritis, two neuroborreliosis, and four non-specified cases), and 7% putative persisting *B. burgdorferi* infection – or preceding LD [21% post-treatment LD syndrome (PTLDS)]. Using logistical regression analysis, OR's predictive for current LD were calculated for arthralgia [3.768 (1.046–13.566)], tingling sensations [0.133 (0.023–0.785)], fatigue [0.169 (0.45–0.632)], female gender [0.235 (0.067–0.825)], duration of symptoms in weeks [0.994 (0.990–0.999)], and recollection of tick bites [8.922 (2.047–38.887)]. Of the 51% without LD, 23% was explained by another diagnosis, mostly osteoarthritis or allergy/eczema, but incidentally by multiple sclerosis, paraneoplastic syndrome, polymyalgia rheumatica, or delusional parasitosis. We treated 25% of patients with recommended antibiotic therapy. In 65% of the patients, we were informed about the clinical course and subjective improvement was reported by all local and early LD and PTLDS patients. However, non-, late and putative persisting LD patients improved in, respectively, 53, 57 and 67%.

Discussion: In agreement with others, we confirm that the majority of referred patients had no current LD and patients with local and early LD respond well to antibiotic treatment. PTLDS patients reported improvement over time without additional antibiotic retreatment. Interestingly, 50% of PTLDS patients received additional antibiotic retreatment before referral. We will continue evaluating patients referred to the AMLC to determine the burden of LD.

B062 (Ep014)

Human *Borrelia miyamotoi* infection in the United States

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Introduction: *Borrelia miyamotoi*, a spirochete that is genetically related to the species of *Borrelia* that cause relapsing fever, has been detected in all tick species that are vectors of Lyme disease. It was detected in *Ixodes scapularis* ticks from Connecticut in 2001 and subsequently has been found in all areas of the United States where Lyme disease is endemic, including 15 states in the northeast and northern Midwest and in California. The first human cases of *B. miyamotoi* infection were reported in central Russia in 2011. We now provide evidence of human *B. miyamotoi* infection and prevalence of this infection among people in the United States.

Methods: Enzyme-linked immunosorbent assays and confirmatory Western blot assays of archived serum samples obtained from three groups of patients, who were living in areas where Lyme disease was endemic between 1990 and 2010, were used to detect antibody against *B. miyamotoi* GlpQ protein (an antigen that is nonreactive to *Borrelia burgdorferi* antibody). Group 1 consisted of 584 healthy subjects from southern New England, who participated in serologic surveys for tick-borne infections. Group 2 included 277 patients from southern New England, who were evaluated for suspected Lyme disease. Group 3 consisted of 14 patients from southern New York, who were evaluated at a Lyme disease clinic with a viral-like illness in the late spring or summer.

Results: The *B. miyamotoi* seroprevalence among healthy subjects (group 1) was 1.0%. Three patients who experienced a viral-like illness had a *B. miyamotoi* GlpQ seroconversion where the antibody titer was at least four times as high in the convalescent serum samples as in the acute serum samples and confirmed by Western blot assay. Two of these patients were co-infected, one with Lyme disease and one with babesiosis. One patient, who had no erythema migrans skin lesion and no laboratory evidence of human granulocytic anaplasmosis co-infection, experienced a fever of 39.4°C, chills, sweats, a headache, neck stiffness, fatigue, myalgias, arthralgias, abdominal pain, cough, sore throat, and right inguinal lymphadenopathy. The other two patients also presented with a viral-like syndrome. All patients became asymptomatic after treatment with doxycycline.

Conclusion: These data suggest that *B. miyamotoi* infection may be prevalent in areas where Lyme disease is endemic in the United States and can be associated with viral-like symptoms. Additional research is needed to determine the frequency of infection, spectrum of clinical symptoms, and most appropriate diagnostic testing and treatment for this newly appreciated infection.

B063 (Ep015)

Immobilization, agglutination, and lysis of *Borrelia burgdorferi* spirochetes in horse serum: implications for diagnosis of equine borreliosis

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Equine borreliosis is poorly understood and substantial controversy surrounds its existence. A number of vertebrate species are innately immune to the Lyme disease (LD) agent, *Borrelia burgdorferi* and inclusion of horses in this group might reduce the number of false positive equine LD diagnoses. To determine if horses display innate immunity to *B. burgdorferi* and if the same immunological mechanisms that exist in other species for this pathogen are likely to be important in horses, we conducted *in vitro* assays of live *B. burgdorferi* cultures incubated with and without horse serum and analyzed rates of spirochete immobilization, lysis, and agglutination. To identify potential mechanisms of borreliacidal activity, we used equine sera with functional complement as well as those in which complement pathways had been heat-inactivated. We quantified changes in spirochete viability in both heat-treated and untreated sera by linear regression. Spirochetes incubated in horse serum suffered significant mortality over time (>80% within 4 h) when compared to control samples incubated in the absence of horse sera. We detected no difference in borreliacidal activity between heat-treated and untreated sera suggesting that the complement system is not responsible for agglutination and lysis of *B. burgdorferi* spirochetes. As a point of reference, we conducted identical assays with white-tailed deer (*Odocoileus virginianus*) serum; heat-treated deer sera failed to kill substantial numbers of spirochetes but untreated serum was hostile to *B. burgdorferi*, a finding which is consistent with xenodiagnostic analysis showing white-tailed deer to be non-competent hosts for this pathogen. Our finding that horse serum is apparently hostile to *B. burgdorferi* spirochetes is consistent with low numbers of confirmed clinical diagnoses of equine borreliosis and suggests that horses might have a relatively high level of innate immunity to this pathogen.

B064 (Ep016)

Risk of Lyme borreliosis and human exposure to ticks of chosen Landscape Park in South Poland

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Lyme borreliosis (LB) is the most common vector-borne disease in Europe. LB is caused by spirochete *Borrelia burgdorferi* sensu lato (BBsl), which is transmitted to humans by ticks, in Europe and Poland mainly the species *Ixodes ricinus* – commonly called the sheep tick or castor bean tick. The objective of this study was determination of the risk of human exposure to vector ticks and LB during high tourist season in recreational area (Zywiec Landscape Park) in the populous Zabnica region of South Silesia. Four different places were sampled (picnic areas, hunting tree stands areas, hiking trails, forest workplaces) by dragging a woolen flag over lower vegetation in June and September 2012. Human-biting ticks were enumerated (n = 198) along all trails: *Ixodes ricinus* (100%), including females (14.14% of total), males (16.16% of total), nymphs (68.18% of total), and larvae (1.52% of total). Sixty ticks were randomly selected with equal to number of each tick stages, from the above-mentioned study places. All *I. ricinus* ticks were investigated individually. Bacterial DNA was isolated by ammonium hydroxide method. The isolates obtained from *I. ricinus* ticks were examined for the presence of BBsl by PCR using primers FLA1 and FLA2 specific for fragment of the *fla* gene sequence. Only 1/60 (1.66%) of examined ticks pathogen was detected. The study showed high risk of human exposure to ticks and presumably there is no risk of LB in forest workplaces where BBsl (1.66%) was detected. Future studies should be continued by taking into account the spring season, the other regional parks in South Poland and incorporate tests for multiple pathogens.

B065 (Ep019)

Study on Lyme neuroborreliosis in Transylvania, Romania

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Introduction: Lyme neuroborreliosis (LNB) may mimic almost any neurological disease and patients suffering from other neurological disorders may be misdiagnosed with Lyme borreliosis (LB).

Aims: Although in the Clinic of Infectious Diseases, Cluj-Napoca, Romania, patients are treated for LNB, no study has been performed so far following the European diagnostic criteria that recommend CSF analysis. We have addressed the question if the apparent poor response to antibiotic treatment in our patients is due to an incorrect diagnosis.

Patients and Method: A prospective study included hospitalized patients with the suspicion of LNB between March 2011 and October 2012. Inclusion criteria were central nervous system manifestations, positive serology for *B. burgdorferi*, lumbar puncture, and no previous parenteral antibiotic treatment. A questionnaire was completed for each patient by a medical investigator regarding previous or present co-existing diseases, tick bites history or erythema migrans, neurological, musculoskeletal, cutaneous, cardiac and ocular signs, and symptoms at admission, at the end of treatment and 3 month later. Two-tiered testing was used for screening Genzyme Virotech ELISA kit in 2011 and Euroimmun ELISA kit in 2012, followed by EUROLINE-RN-AT Western blot (Euroimmun). The intrathecal antibody index (IAI) was calculated, using a Genzyme Virotech GmbH, Germany kit in 2011 and an Euroimmun AG, Germany kit in 2012. Patients were treated as LNB with parenteral therapy (ceftriaxone 2 g/day or cefotaxime 6 g/day in case of cholelithiasis) for 21 days, indifferent of the CSF analysis results. Clinical and serological evaluation (ELISA) was repeated 3 month post-treatment. Statistical analysis used Fisher exact test and Poisson mixed regression models.

Results: Forty-two patients were included and epidemiological, clinical, and serological data of the patients will be presented. According to European guidelines no case fulfilled the criteria for definite LNB, 7 cases were possible LNB and in 33 cases LNB was infirmed. Two cases could not be classified due to insufficient amount of CSF. Adverse reactions to medication were present in 13 patients, including one *Clostridium difficile* associated disease. The evolution of signs and symptoms in possible/infirmed LNB subgroups will be presented.

Conclusion: (1) This study brings evidence that LNB is over diagnosed in our patients; (2) It is the first proof to stop the antibiotics overuse in not confirmed LNB patients in our region.

B066 (Ep022)

Geographic and genospecies distribution of *Borrelia burgdorferi sensu lato* strains detected in humans in the United States

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The present study sought to determine the cause of Lyme-like illness inhuman patients primarily in the southern U.S. with suspected Lyme disease based upon EM-like skin lesions and/or symptoms consistent with early or late stage Lyme borreliosis; the study also included patients from other states throughout the USA. Using several polymerase chain reaction assays (PCR) specific for either *Borrelia* spp. or only for Lyme group *Borrelia* spp., followed by DNA sequencing for confirmation, we identified *Borrelia burgdorferi* sensu lato DNA in blood and skin biopsy samples from patients residing in southern states and elsewhere in the U.S., but no evidence of *Borrelia lonestari* infection. PCR and DNA sequence data showed that *B. burgdorferi* sensu stricto, *Borrelia. americana*, and *Borrelia. andersonii* appear to be associated with Lyme disease-like signs and symptoms in patients from southern states, as well as some states in other regions of the country. Based on these findings, we suggest that human cases of Lyme-like illness often referred to as STARI in the southern U.S. or given other diagnoses may be attributable to previously undetected *B. burgdorferi sensu lato* infection.

B067 (Ep023)

Lyme borreliosis in human patients in Florida and Georgia, USA

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The present study sought to determine the cause of Lyme-like illness in several human patients residing in Florida and Georgia, USA, with suspected Lyme disease based upon EM-like skin lesions and/or symptoms consistent with early or persistent Lyme borreliosis. Using polymerase chain reaction assays (PCR) for either *Borrelia* spp. or specifically for Lyme group *Borrelia* spp., followed by DNA sequencing for confirmation, we identified *Borrelia. burgdorferi* sensu lato DNA in blood, skin biopsy samples, or attached lone star ticks (*Amblyomma americanum*) from several patients residing, or with tick exposure history, in Florida or Georgia. This report presents and discusses the first specific molecular evidence of Lyme *Borrelia* spp. infection in human patients in the southern U.S., including several following lone star tick bites. Furthermore, the evidence shows that several *B. burgdorferi* sensu lato species may be associated with Lyme disease-like signs and symptoms in patients from southern states. Based on the findings of this study, we suggest that human Lyme borreliosis occurs in Florida and Georgia, and that cases of Lyme-like illness often referred to as STARI in the southern U.S. may be attributable to previously undetected *B. burgdorferi* sensu lato infections.

B068 (Ep024)

Risk factors associated with seropositivity against Lyme borreliosis: results from a representative serosurvey of adults in Germany

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Background: Lyme borreliosis (LB), caused by *Borrelia burgdorferi* (Bb) sensu lato is the most common tick-borne disease of the northern hemisphere. Major clinical manifestations include Erythema migrans, Neuroborreliosis and Lyme arthritis. Central Europe including Germany is regarded a highly endemic area; however, data on the extent of endemicity and on risk factors are limited.

Objectives: Our objectives were to conduct a representative seroepidemiological survey among adults in Germany to assess the seroprevalence of LB indifferent geographical regions and to identify potential risk factors for seropositivity.

Methods: Sera from a nationwide representative survey for adults (DEGS) (2008–2011) were analyzed by ELISA to assess the prevalence of anti-Bb IgG-antibodies in adults (18–79 years). An immunoblot was carried out as a confirmatory test. Data collected by interview were used to evaluate possible associated factors. Univariable and multivariable statistical analyses used sampling weights and accounted for the cluster structure of the survey design.

Results: Out of a total of 6,965 individuals 741 were seropositive. Seroprevalence in men [13.0%; 95% confidence interval (95%-CI: 11.4–14.8%)] was twice as high as in women (5.8%; 95% CI: 4.9–6.7%). Seropositivity increased from 6.0% in the 18–29-year-age-group to 20.0% in the 70–79-year-age-group without effect modification by sex. The largest increase was observed in the elderly (60–79 years). Residence in rural areas [Odds ratio (OR) = 2.50; 95% CI: 1.85–3.30] and living in Southern Germany (OR = 1.43; 95% CI: 1.08–1.88) were independent risk factors for seropositivity.

Conclusions: LB is endemic in all over Germany. The seroprevalence seen in the different age-groups reflects the cumulative incidence proportion. A previous study recognized children and adolescents (1–17 years) as a risk-group, this study identified elderly (60–79 years) as a second risk-group. This is compatible with recreational behavior. Alternatively, this might be explained by a birth cohort effect of a previous time frame of lower risk of seroconversion in Germany resulting in less increase in 18–59 year-old. Seropositivity is not equivalent with clinical manifestation. Thus, differences between groups may not necessarily be reflected by the real disease burden. Identified risk factors are useful for targeted campaigns. Furthermore, the knowledge on seroprevalence in the population is a basic requirement to adjust pre-test probabilities in context of clinical diagnoses.

B069 (Ep025)

Environmental proxies for tick density and human behavioral risk factors for Lyme disease infection on block Island, Rhode Island

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Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is the most commonly reported vector-borne disease in the US, with >20,000 cases reported annually. The black-legged tick, *Ixodes scapularis*, serves as the principal vector in maintaining the spirochete's zoonotic transmission, as well as transmission to humans. Human risk of infection with tick-borne pathogens is determined primarily by the interaction between environmental or "acarological" risk (density of infected *I. scapularis* nymphs that are actively host-seeking) and human behaviors increasing exposure risk to infected ticks. The goal of this study was to assess the relative significance of acarological risk and protective behaviors in reducing Lyme infection risk. To assess behavioral modifiers of risk, we examined data from a longitudinal cohort study of 1132 records of Block Island residents who participated in biannual serosurveys from 2005 to 2011. We identified remotely sensed landscape characteristics of people's yards, which were associated with higher density of *I. scapularis* nymphs and used these metrics as proxies for infection exposure risk. Using a negative binomial regression, we found statistically significant positive unadjusted associations between positive Lyme serology and a number of landscape metrics, hours spent outdoors, and owning a cat that spends time indoors and outdoors; using protective clothing was found to be protective. Based on these findings, we constructed a multivariate logistic regression model including wearing protective clothing (protective) and occupational exposure, hours spent in tick habitat, and the total property edge consisting of shrubs (increasing risk). Results from this study suggest that both environmental risk exposure to ticks and human behaviors should be examined in concert in risk assessment studies and to design optimal interventions.

B070 (Ep028)

Risk of Lyme borreliosis and human exposure to ticks of chosen Landscape Park in South Poland

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Lyme borreliosis (LB) is the most common tick-borne disease in Europe. LB is caused by spirochete *Borelia burgdorferi* sensu lato (Bbsl), which is transmitted in Europe and Poland mainly by the castorbean tick *Ixodes ricinus* (Ir). The objective of the study was to determinate the risk of human exposure to ticks and Bbsl during a high tourist season on recreational areas of the Żywiec Landscape Park, South Poland. A total of 198 (n = 198) Ir ticks were collected from picnic areas (n = 52, 26.26% ticks), hunting blind places (n = 44, 22.22%), hiking trail (n = 34, 17.17%), and forest workplace (n = 68, 34.34%) by flagging lower vegetation in June and September 2012, including females (14.14% of total), males (16.16%), nymphs (68.18%) and larvae (1.52%). Next, 60 ticks were randomly selected with equal to number of each tick stage, from the above-mentioned study places. All ticks were investigated individually. Bacterial DNA was isolated by ammonium hydroxide method. The isolates obtained from these ticks were examined for the presence of Bbsl by PCR using primers FLA1 and FLA2 specific for the fragment of fla gene sequence. Among 60 examined ticks, only one female found in "forest work place" was positive for Bbsl (1.66%). The study showed high risk of human exposure to ticks in all studied areas. Possibility of the Bbsl infection at the forest work place, where positive Bbsl was found, is higher than on other studied places, where tested ticks were negative for Bbsl. Simultaneously, we cannot ascertain that risk of Bbsl infection at the Zywiecki Landscape Park is possibly the same at different areas not included into this study. Wherefore, these surveys should be continued taking into account other seasons of the year and other regional parks of South Poland.

B071 (Ep029)

Ecological and climatic conditions matched with human cases of *Borrelia miyamotoi* infection in Russia

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The main clinical characteristics of a "new" borreliosis caused by *Borrelia miyamotoi* in Russia previously have been reported (Platonov et al., 2011). The objective of this presentation is to describe ecological conditions favoring this infection.

Methods: Acute *Borrelia miyamotoi* cases were identified as previously described (Platonov et al., 2011). Patients self-reported the date and location of tick bite within about 1–3 km. The ecological and climatic conditions in *B. miyamotoi* infection locations (BMIL) were estimated from satellite remote sensing and meteorological observations using the VEGA web-based monitoring system developed by the Space Research Institute.

Results: We analyzed 77 human cases of *B. miyamotoi* infection diagnosed in the Republic of Udmurtia in 2010–2012. All but three BMIL were between 56°N, 58°N, 52°E, and 54°E. Most BMIL were inside the area of 56°30¢ N, 57°15¢N, 52°45¢E, and 53°30¢E, with the center in Izhevsk City. Although there are numerous farms and meadows in Udmurtia, this area is covered mainly by forests. BMIL were surrounded by deciduous forest (36%), coniferous forest (11%), or mixed forest (51%). Sixteen BMIL in Izhevsk were in city parks with mixed forest. Patients were bitten by *Ixodes persulcatus* ticks between May 1 and July 30 (from 18th to 27th weeks of an year) with a peak in 22nd week (18 cases). In this period, the mean daily temperature (MDT) and the Normalized Difference Vegetation Index (NDVI) varied from 5 to 25°C (median 16°) and 0.57 to 0.90 (median 0.82), respectively. Sixty seven (87%) of 77 *B. miyamotoi* cases occurred when MDT was higher than 10°C and the NDVI was more than 0.68. Only 10 of 77 days of infection occurred when precipitation was more than 0.6 g/cm^2 with a relative humidity >80%. We also analyzed 39 human cases of *B. miyamotoi* infection diagnosed in Sverdlovsk Region in 2009. All but two BMIL were inside the area 56°20¢N, 57°20¢N, 60°00¢E, and 61°30¢E, with the center in Yekaterinburg City. BMIL were surrounded by deciduous, mixed, or coniferous forest (7, 24, and 67%, respectively). Twelve BMIL in industrial Yekaterinburg were in city parks/forests. *B. miyamotoi* infections occurred between 19th and 28th weeks of an year with a peak in 22nd week (seven cases). The MDT varied from 3 to 26°C (median 16°C) and the NDVI from 0.55 to 0.87 (median 0.75).

Conclusion: Similar ecological conditions exist where *B. miyamotoi* is endemic.

B072 (Ep030)

Accuracy of reporting of Lyme disease in Connecticut

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Background: Connecticut has a consistently high incidence of Lyme disease. The Connecticut Department of Public Health implemented a system of active surveillance for Lyme disease in selected medical practices, but the accuracy of reporting is unknown.

Objective: To assess the accuracy of diagnosis of Lyme disease among cases reported through the active surveillance case reporting system in Connecticut.

Methods: Study patients were persons who were reported to the Connecticut Department of Public Health as having Lyme disease through an active surveillance case reporting system between January 2000 and June 2003. Information in the active surveillance case report forms was reviewed. Medical records of all subjects were also reviewed.
All reported cases were classified into the following categories: definite Lyme disease, cases that met the national surveillance case definition for Lyme disease; possible Lyme disease, cases that met most criteria but were missing some information (e.g., size of the erythema migrans rash); unlikely to have Lyme disease, all others.

Results: Of the first 680 cases that were reviewed, based on the active surveillance case reports 666 (98%) had definite Lyme disease, 4 (<1%) had possible Lyme disease, and 10 (2%) were unlikely to have Lyme disease; however, based on reviews of the medical records, only 444 (65%) had definite Lyme disease, 16% had possible Lyme disease, and 19% were unlikely to have Lyme disease ($P < 0.001$).

Conclusion: We conclude that even with an active surveillance system for Lyme disease in a highly endemic area, misdiagnosis of the disease in reports to a state Department of Public Health is common.

B073 (Ep032)

The emergence of Lyme disease in China

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More than 20 years since its description, the occurrence of Lyme disease has been confirmed in 30 provinces of China. The etiologic agent has been identified and grown, molecular diagnostic methods have been developed, and the epidemiology and ecology were understood sufficiently to establish avoidance and prevention strategies. In this review, we integrate the information about recent diagnosis methods, epidemiological investigation, and disease distribution in China. It will provide the measures for the control and prevention of Lyme disease in China and to make advantageous wizard for neighboring countries and the world.

B074 (D006)

Laboratory testing for tick-borne diseases in the United States: results of a TickNET survey

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Background: Little is known about current laboratory practices and testing volume for Lyme disease and other tick-borne diseases. A CDC TickNET assessment of major clinical laboratories in the United States (U.S.) was conducted to better understand laboratory practices and testing burden for eight tick-borne diseases in order to better inform public health surveillance and practice.

Methods: Paper-based surveys were sent to the 10 major commercial clinical laboratories most frequently identified in tick-borne disease surveillance records. Each laboratory was asked to report 2008 national laboratory testing volume data for eight tick-borne infections in the U.S.: Lyme disease, rocky mountain spotted fever, babesiosis, anaplasmosis, ehrlichiosis, tick-borne relapsing fever, Colorado tick fever, and Powassan virus.

Results: Seven (70%) large commercial laboratories in the U.S. reported testing volume for tick-borne diseases. All seven responding laboratories reported testing specimens for Lyme disease and babesiosis, six (86%) laboratories reported testing for anaplasmosis, ehrlichiosis, and rocky mountain spotted fever. Two (29%) laboratories reported testing for tick-borne relapsing fever, and one (14%) tested for Colorado tick fever. No commercial laboratories reported testing for Powassan virus. Responding laboratories tested 2,927,881 specimens for tick-borne infections. Tick-borne disease diagnostic tests were conducted most commonly for Lyme disease (83%), followed by ehrlichiosis (6%), rocky mountain spotted fever (5%), babesiosis (3%), anaplasmosis (2%), tick-borne relapsing fever (<1%), and Colorado tick fever (<1%).

Discussion: As Lyme and other tick-borne diseases are likely underreported by physicians in endemic states, laboratory testing data may more accurately estimate the incidence of laboratory confirmed tick-borne disease in the U.S. In addition, laboratory surveillance may present a more sustainable methodology for tracking U.S. tick-borne diseases. However, since positive tests may only indicate seropositivity, it is yet unclear how many confirmed and probable incident tick-borne disease cases could truly be ascertained through laboratory surveillance alone. Nevertheless, these results provide baseline test utilization data for continued evaluation of tick-borne disease testing trends in the United States.

B075 (D013)

Serological diagnosis of Lyme borreliosis in suspected patients in Korea, 2008–2012

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Lyme borreliosis (LB) is a tick-borne disease caused by *Borrelia* spp. Since 2010, LB has been designated as a legal infectious disease in Korea. In this study, we collected 3,296 samples (sera and CSF) from LB suspected patients to investigate LB infection and incidence during 2008–2012 in Korea. We performed indirect immunofluorescence assay (IFA) as a screening test and Western blot as a confirmatory test. Total 1,299 (39.41%) cases were seropositive by IFA, and 32 (0.97%) cases were positive by Western blot. Western blot assay can detect IgG and IgM antibodies directed against *B. burgdorferi* using recombinant antigens of *B. burgdorferi, B. afzelii*, and *B. garinii* like p100, p41, p39, p18, OspC, OspA, and VlsE. The main symptoms were showed neuropathy, fever, atrophoderma, and erythema migrans. Among the positive results by Western blots, 13 cases were infected abroad and the remaining 19 cases suspected to have been infected in Korea. The isolation of *Borrelia* spp. from human has not yet been reported in Korea. However, based on cases of LB diagnosed serological diagnosis, broad and active surveillance program is necessary to prevent continuous occurrence of LB in Korea.

B076 (D018)

Lyme neuroborreliosis is highly prevalent in tertiary care hospitals of Central-southeast Mexico

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Lyme disease is considered the number one disease transmitted by tick bite worldwide, and is a multisystemic disease where neurologic manifestations may cause severe disabling episodes. In 2011, the CDC reported more than 24,000 confirmed cases with neurological manifestation 14%. In Mexico, the presence of Lyme disease has been documented since 1999, infected vector and studies conducted in patients with neurologic manifestations of Guillain–Barre syndrome and facial palsy found an infection rate of 14 and 12.5% respectively. Currently, there are no reports on the frequency and diversity of neurologic cases attributed to Lyme disease in Mexico.

Objective: To determine the prevalence of Lyme Neuroborreliosis in tertiary care hospitals of Instituto Mexicano del Seguro Social (IMSS) attending patients from central and southeast Mexico.

Materials and Methods: Cross-sectional study made in the period from 1999 to 2011, and approved by the ethics committee of IMSS. Blood and cerebral spinal fluid (CSF) samples were taken from patients with neurological symptoms suggestive of Lyme disease according to the CDC criteria (ELISA and Western blot IgG+ vs. *Borrelia burgdorferi* in serum and/or positive in CSF). Results were analyzed with chi square, Fisher exact test, *U* Mann–Whitney, and student *T* test.

Results: We studied 650 patients and confirmed Lyme Neuroborreliosis in 132 (20.3%). The acute disseminated stage was documented in 100 (75.7%) with facial palsy (38), lymphocytic meningitis (35), poliradiculopathy 27 cases, and the chronic stage in 32 (24.3%) of the cases with encephalomyelitis. Were 91 adults (67%) aged 42 ± 14.7 years and 44 children (33%) aged 9.6 ± 5 years, and a sex ratio male: female 1:1.3. In 28/132 cases (20.7%) presented tick bite and four of the negatives OR 5.2 (95% CI: 3.2–10.6 $p < 0{,}001$). Among the neurological signs, predominant facial palsy unilateral, paresthesias ($p < 0.05$; OR 3.6, 95% CI: 1.39–9.49), distal weakness ($p < 0.001$; OR 14.7, 95% CI: 7–32), and pleocytosis in CSF ($p < 0.05$) were more frequent in cases.

Conclusions: This is the first report of Lyme neuroborreliosis in central of Latin-American, and presents as disseminated and a chronic stage. The scientist and the physician must consider that LD is distributed more extensive in America. Only the obligatory report by the Public Health ministry in Latin-America will define the real impact.

B077 (D025)

Prevalence of tick-borne pathogens field collected ticks from the United States and Europe

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Background: Tick-borne diseases, many of which have similar symptoms, are caused by a wide range of bacteria, protozoa, and viruses, and can be transmitted by the same vector. Active surveillance of ticks can provide health agencies with data for population risk assessments and inform physicians as to the possible pathogens patients may encounter. Performing risk-assessment and determining the prevalence of the pathogens previously required a specific test for each distinct pathogen resulting in costly and time-consuming processes, which could not always detect novel species or strains. In this ongoing long term study, we demonstrate the use of multi-locus broad-range PCR and electrospray ionization mass spectrometry assays (PCR/ESI-MS) to detect numerous pathogens as well as various strains of *Borrelia burgdorferi* from ticks across the United States and Europe.

Methods: We used a multi-locus broad-range PCR/ESI-MS assay designed to detect a wide range of vector-borne bacterial, viral, and protozoal pathogens. Additionally, we utilized a more specific assay for genotyping *Borrelia*. Total nucleic acids were extracted from ticks collected at various sites across North America and Europe and screened on both the assays. Electrospray ionization mass spectrometry of the PCR amplicons was used to determine their base composition. These base composition signatures were subsequently used to identify the organisms found in the samples.

Results: We examined field collected ticks from the United States and Europe for pathogens and potential pathogens. We found numerous pathogens present both as single infections and co-infections in these ticks including bacterial, viral, and protozoal co-infections. In instances where *Borrelia* was detected, we often got a genotype signature and in many instances could determine that multiple strains of *Borrelia* were present as a co-infection in the tick. We will show data from hundreds of ticks from various species of *Ixodes, Dermacentor*, and *Amblyomma* ticks.

Conclusion: We have demonstrated here broad range detection of tick-borne pathogens and potential pathogens PCR/ESI-MS. This has use in public health surveillance for risk assessment of potential vector-borne disease as well as potential applications for animal health and clinical diagnostics.

B078 (D040)

Investigation of Lyme disease in China during 2010–2012 diagnosis and distribution

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Lyme borreliosis, caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato group, and transmitted through *Ixodes* ticks. Because there is currently no vaccine available to prevent Lyme borreliosis in humans, early diagnosis and prevention is particularly important, one should be aware of atypical presentations and be able to formulate an efficient and effective diagnosis plan. In this update, we will discuss the diagnosis of *Borrelia burgdorferi,* the epidemiology of its vectors and hosts, the transmission of Lyme disease, and the distribution of *Borrelia burgdorferi* in China during 2010–2012.

B079 (M001)

Genoespecies of *Borrelia burgdorferi* in ticks of the Northeast of México

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Lyme disease represents the most frequent tick-borne infectious disease in the United States and Europe (1). The incidence of human Lyme disease correlates with the prevalence and abundance of *Ixodes* ticks infected with *Borrelia burgdorferi* sensu lato in the region (2). *Ixodes scapularis* and *I. pacificus* are the competent vectors of *B. burgdorferi* in the US but, Recently other species of infected ticks are reported in Texas and the Northeast of Mexico. Since 1999, we reported a seroprevalence of 1.1% to *B. burgdorferi* in general population of Mexico with prevalence of 6.4% in the states of the Northeast border with Texas, where we also reported the presence of *Ixodes* ticks infected with *B. burgdorferi* sensu stricto. Clinical cases with cutaneous and neurological manifestation reported exclusively in Europe have been reported in Mexico, but unknown if the species of *B. burgdorferi* is more diverse that reported in North America.

Objective: In this study we aimed to search the genospecies of *B. burgdorferi* that infected ticks of the Northeast of Mexico.

Materials and Methods: We selected *Ixodes* ticks preserved in ethanol of a collection of Emerging Infectious Disease laboratory, UIMEIP, Centro Medico Nacional SXXI, IMSS. The ticks were collected on mammals and plants from 18 forest and rural localities of Tamaulipas and Nuevo Leon, border states with Texas during the period 1990–2007. Infections were determined in extracted DNA by PCR amplification with the Fla B, OspA and intergenic 23S genes for *B. burgdorferi.* The positives ticks were sequencing to genotyping.

Results: Were positive for *B. burgdorferi* 36 (10.2%) ticks of eleven localities near of three big cities. All bacteria were identified how *B. b.* sensu stricto with homology of 96–100%. The infected ticks were identified as *I. scapularis* 4 (1.1%), *Dermacentor andersoni* 4 (1.1%), *Amblyomma americanum* 2 (0.6%) *Rhipicephalus sanguineus* 14 (4%), *Amblyomma cajennense* (11 3.1%), and *Boophylus microplus* (1.1%).

Conclusions: *B. burgdorferi* sensu stricto was prevalent in ticks of the Northeast of Mexico. *R. sanguineus* and *A. cajennense* were the ticks frequently infected, although its role as a competent vector is unknown. Is possible that potential vectors are more diverse in Mexico and Texas. The physician must consider Lyme disease in the differential diagnosis from these endemic areas.

B080 (M011)

Investigation of the *Borrelia* content in ticks that bites humans

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Lyme borreliosis (LB) risk assessments and epidemiology has previously been studied by investigating *Borrelia* prevalence in field-collected ticks. However, more accurate risk assessments and epidemiology would be achieved if ticks that actually bite humans were investigated. The risk of acquiring a LB infection increases with time of tick feeding. However, the knowledge about the impact of spirochetal load in a feeding tick is limited. Therefore the present tick-borne diseases (TBD) Sting study aimed to explore the prevalence and phylogeography of *Borrelia* spirochetes in *Ixodes ricinus* collected from newly tick-bitten humans from highly endemic areas for LB and from areas with unknown LB status in Sweden and at the Åland Islands, Finland. Ticks were identified morphologically to determine species, life stage, sex, and estimated feeding-time by using aUSB-microscope. The ticks were homogenized and total nucleic acids were extracted. cDNA was analyzed with a LUX real-time PCR assay to detect and quantify the *Borrelia* 16S rRNA gene. *Borrelia* species in positive samples were determined by a nested, conventional PCR assay using primers targeting the non-coding intergenic spacer regions (IGS) 5S–23S or the 16S–23S IGS rRNA genes, respectively. A total of 2,154 ticks, including 15 adult males, 496 adult females, 1,510 nymphs, 87 larvae were collected. All ticks were determined as *Ixodes ricinus*, except for 46 undetermined ticks. Approximately one-fourth of the collected ticks harbored *Borrelia* spirochetes. Adult ticks had a higher *Borrelia* prevalence (36%) compared to nymphs (25%, $p < 0.001$). No *Borrelia* was detected in any of the larvae. PCR sequencing identified seven different *Borrelia* species with *Borrelia afzelii* as the predominant species in the collected ticks followed by *Borrelia garinii, Borrelia valaisiana, Borrelia burgdorferi* sensu stricto (s.s.), *Borrelia miyamotoi, Borrelia spielmanii*, and *Borrelia lusitaniae*. The median number of spirochetes per tick ranged was 1.6 ´ 103. Ticks that were infected with *B. miyamotoi* harbored more spirochetes (median of 2.1 ´ 105) than ticks infected with *B. afzelii, B. garinii, B. valaisiana*, and *B. burgdorferi* s.s., among ticks that had fed for ~ 24 h. In conclusion, one-fourth of the ticks that had bitten humans in Sweden and at Åland Islands were infected with *Borrelia*. The *Borrelia* species *B. spielmanii, B. lusitaniae* and *B. miyamotoi* were, to our knowledge, detected for the first time in ticks detached from humans, in Sweden and at the Åland Islands.

B081 (M017)

Molecular detection and identification of *Borrelia burgdorferi sensu lato* in rodents in Slovenia

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Objective: *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, is mainly maintained in natural foci through transmission cycles of competent tick vectors and vertebrate reservoir. Specific rodents were identified as the principal reservoir of *Borrelia burgdorferi* sensu lato in Europe. In the present study, molecular epidemiological survey was performed to determine the presence of *Borrelia burgdorferi* sensu lato in rodents present in seven different geographic area of Slovenia.

Materials and Methods: A total of 386 rodents were live-trapped. After identification of species, organs were removed and stored at -80°C until tested. DNA from lung was isolated and the presence of *B. burgdorferi* sensu lato DNA was determined using two different PCRs, "in house" nested and commercial real-time PCR (LightMix® Kit for detection of *Borrelia* spp., TIB MOLBIOLGMBh, Germany) both targeting ospA gene. Identification of *Borrelia* species was done by melting temperature (Tm) analysis included in LightMix® protocol and by sequencing of PCR product of "in house" PCR.

Results: Borrelial DNA was detected in 41/386 (10.6%) and 13/386 (3.4%) lungs with LightMix® and "in house" PCR, respectively. Concordance of both tests was 93%. Prevalence of *Borrelia burgdorferi* sensu lato infected rodents differed regarding to particular geographic region and ranged from 0 to 30% according to LightMix® and 0–15% according to "in house" PCR. Identification according to Tm using LightMix® revealed the presence of *Borrelia afzelii* or *Borrelia valaisiana*, while analysis of Tm does not distinguish between these two species. Sequencing of "in house" PCR products revealed the presence of *B. afzelii*, only.

Conclusion: Prevalence of with *Borrelia burgdorferi* sensu lato infected rodents depends on geographic region and used molecular test; it ranges from 0 to 30%. LightMix® was found to be more sensitive in comparison to "in house" PCR. Although LightMix® does not allow distinction of *B. afzelii* and *B. valaisiana,* sequencing of "in house" PCR products of ospA gene reveals presence of *B. afzelii* only, which is in concordance with the results from the literature.

B082 (M018)

Molecular typing of flab gene of a *Borrelia garinii* 935T isolated from *Ixodes persulcatus* in Korea

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Lyme borreliosis (LB) is a tick-borne zoonotic disease caused by genus *Borrelia*. *Borrelia burgdorferi* sensu lato complex, the main pathogen group of the disease, is composed of 11 species including *B. burgdorferi* sensu stricto, *Borrelia afzelii, Borrelia garinii, Borrelia andersonii, Borrelia bissenttii, Borrelia joponica, Borrelia valaisiana, Borrelia lusitaniae, Borrelia tanukii, Borrelia turdi, and Borrelia sinica.* Genome of *B. burgdorferi* sensu lato has one linear chromosome and 17–21 plasmids. Differences in genetic features 16S rRNA, ospA, flaB, noncoding genome rrfA-rrlB, and rrs-rrlA for intergenic regions have been used for distinct identification of *Borrelia* species. flaB encoding flagellin is a major antigen inducing strong IgG and IgM immune responses in early infection. Because flaB has a genetic variation, it has been considered as a target gene for identification of *Borrelia* species by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using HpyF31 and Ecll36II restriction enzymes. In Korea, *B. garinii* 935T strain was isolated from *Ixodes* persulcatus in 1993. The protein profiles and reactivities to monoclonal antibodies for FlaB, OspA and OspB were analyzed in this strain. In this study, flaB of the strain was amplified by PCR with 220f/823r primers as previously described and then the amplified DNA was sequenced. The result of PCR-RFLP of flaB clustered V pattern by Beata Wodecka (2011). We will further investigate the characterization of other virulence factors, which may lead to a better understanding 935T strain.

B083 (M025)

Borrelia burgdorferi isolated from *Peromyscus maniculatus* and *Myodes gapperi* in Eastern North Dakota

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Eastern North Dakota is traditionally viewed as a non-endemic area for Lyme disease, though the emergence of confirmed Lyme disease cases among residents suggests eastern North Dakota is minimally a transition zone for *Borrelia burgdorferi* and *Ixodes scapularis.* Indeed, based upon the isolation of infected nymphal *Ixodes scapularis* ticks, Diuk-Wasser et al. (*Am J Trop Med Hyg* 2012 86:320-7), described eastern North Dakota as a transitional zone. In our study, five spirochetes, based on cell morphology, were isolated from the hearts of *Peromyscus maniculatus* and *Myodesgapperi* around the Turtle River and Forest River areas of Grand Forks, ND. The spirochetes underwent not more than six passages *in vitro* before genetic analyses were performed. Sequence analysis of 16S, flaB, ospA, ospC, p66, and the 16S–23S intergenic spacer region (IGS) indicates the spirochetes are *B. burgdorferi*. 16S sequences (ca. 136 nt) aligned to multiple *Borrelia* spp. using the Ribosomal Database Project website. BLAST results and alignments of ca. 40 residues of flaB showed 100% identity to multiple *B. burgdorferi* strains. p66 showed 100% sequence identity across 239 residues to *B. burgdorferi* B31. Two of the five spirochetes were negative for ospA despite multiplex PCR results showing all possessed the plasmid carrying ospA. Mutiplex PCR for 13 linear and 9 circular plasmids revealed a unique complement of linear and circular plasmids among the isolates, suggesting either the arrival of multiple strains of *B. burgdorferi* or rapid divergence of a single strain. All five isolates are missing linear plasmid 25, suggesting they have a low-infectivity phenotype. Future work includes alignment of our eastern North Dakota *B. burgdorferi* sequences with *B. burgdorferi* from western Minnesota to determine directionality and evolutionary history of *B. burgdorferi* in eastern North Dakota; collection of *Ixodes* ticks and comparisons of *B. burgdorferi* to those found in local small mammals; and infection studies to determine the infectivity phenotype of the local isolates. In total, our results confirm that *B. burgdorferi* is present in the small mammal population of eastern North Dakota, further corroborating the eastern progression of *B. burgdorferi*.

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