Abstracts

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The performance of nine commercial serological screening assays for the diagnosis of Lyme borreliosis: a multicenter modified two-gate design study

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Introduction
In this retrospective study, the performance of nine serological screening assays for Lyme borreliosis (LB) diagnostics was evaluated using a study population of LB cases and controls.

Materials and Methods
Sera derived from 74 well-defined LB cases and 122 controls were included. The LB cases were diagnosed with erythema migrans (EM; n=11), Lyme neuroborreliosis (LNB; n=35), Lyme arthritis (LA; n=20) and acrodermatitis chronica atrophicans (ACA; n=8). Controls comprised 74 age- and gender-matched healthy individuals and 48 patients with other diseases with anticipated high rates of cross-reactivity. The assays under evaluation were selected based on a literature review and expected continued availability with CE marking under the new in vitro diagnostic Regulation (EU) 2017/746.

Results
The overall sensitivity (IgG and IgM results combined) among LB cases ranged between 54.5% and 90.9% for EM patients and between 97.1% and 100% for patients with LNB, LA and ACA. The positivity rate ranged between 8.1% and 29.7% among the healthy controls and between 22.9% and 64.6% among the cross-reactivity controls. The IgM assay results were more heterogeneous compared to the IgG and IgM/IgG assay results and did not contribute to the overall sensitivity; however, substantially increased the positivity rates among the controls.

Discussion/Conclusions
In conclusion, all evaluated Borrelia serological screening assays performed comparably with respect to early and late disseminated LB. The addition of an IgM assay to the screening of Borrelia-specific IgG antibodies had no added value for the diagnosis of Lyme borreliosis.
O2. Lost in plasmids continued – The complexity of *Borrelia* plasmid reconstruction

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Abstract:
Bacteria of the *Borrelia burgdorferi* sensu lato species complex are the causative agent of Lyme borreliosis [1] with different genospecies varying in their vector/host associations and human pathogenicity [2, 3]. However, the genetic basis for these adaptations remains unresolved and requires complete genomes for comparative analyses. *Borrelia* genomes include a linear chromosome and > 20 circular and linear plasmids, which have important roles in borrelial adaptation [4]. However, assembling complete *Borrelia* plasmids remains challenging due to the high levels of genome complexity and intra-species plasmid homology. Previous work demonstrated that a combination of short (Illumina) and long read (SMRT, PacBio) sequencing technologies produced superior *Borrelia* plasmid assemblies [5]. Novel technological advancements in long read sequencing (PacBio HiFi) promises very high accuracy data (>99%) [6] – could this represent a solution to the *Borrelia* plasmid assembly problem?

We generated and compared Illumina and PacBio (traditional and HiFi) data for about 30 *Borrelia* isolates and used hybridSPAdes [7] for Illumina read assembly and three different assemblers for PacBio data: Microbial Assembly for traditional PacBio reads, IPA and HiCanu Assembler [8] for PacBio HiFi reads.

Initial results showed that no assembler unequivocally performed best for the entire dataset and not even for all genome elements of one isolate. Depending on assembler and isolate, plasmid(s) were incomplete or not recovered from one assembler but were probably (completely) reconstructed using another assembly strategy. Our results show that through comparing and combining the different assembly strategies for each isolate we could reconstruct completed genomes, including all plasmids.

References:
O3. Discriminating between Lyme neuroborreliosis and other central nervous system infections using Principal Component Cluster Analysis

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Introduction

CXCL13 in cerebrospinal fluid (CSF) is a biomarker for Lyme neuroborreliosis (LNB) (1). The addition of IL-6 in connection with CXCL13 may improve the discriminatory power in separating central nervous system (CNS) infectious diseases (2) (3). This study aims to evaluate the diagnostic performance of CXCL13 and IL-6, in the differentiation between LNB and other neuroinfections.

Methods

The levels of CXCL13 and IL-6 in CSF from patients examined for CNS infections at Lillebaelt Hospital in the Region of Southern Denmark, were quantified using the Bio-Plex 200 System (Bio-Rad). Patients were grouped into definite LNB, possible LNB, viral CNS infection, bacterial CNS infection, Other CNS disease (with pleocytosis) and Negative (without pleocytosis) based on clinical and paraclinical findings.

Results

A total of 390 patients with definite LNB (n=31), possible LNB (n=10), confirmed viral or bacterial CNS infection (n=34), Other CNS disease (n=58), and Negative (n=257) were included. Principal Component Analysis (PCA) revealed three distinct clusters based on leukocyte cell count, protein concentration, CXCL13 and IL-6 concentrations in CSF from 380 included patients (10 possible LNB patients excluded). The clusters were shown (with visual and statistical presentation) to differentiate the groups: definite LNB, bacterial CNS infection and Negative. Among the ten possible LNB patients classified using the cluster model, only three had a profile clearly consistent with LNB. The details of these 10 cases will be presented.

Conclusion

This study confirms that CXCL13 is a supplement for diagnosis of LNB, and that the combination of CXCL13 and IL-6 may be used to distinguish patients with possible LNB from those with other neuroinflammatory conditions.

REFERENCES

**O4. Diagnosis of Lyme neuroborreliosis: What is the diagnostic value of serum *Borrelia burgdorferi* antibodies?**

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**Introduction** Lyme neuroborreliosis (LNB) is diagnosed by a combination of a positive *B. burgdorferi* s.l. intrathecal antibody index test (*Bb*-AI), CSF pleocytosis and clinical symptoms. The correlation between *B. burgdorferi* s.l. serum antibodies (Serum-*Bb*) and the *Bb*-AI remains a subject of debate. ², ³

**Material and methods** We included all Danish residents who had a *Bb*-AI and a corresponding Serum-*Bb* between 1994-2020. We calculated the proportion of individuals with a positive *Bb*-AI who had a negative corresponding Serum-*Bb*-neg. We investigated the proportion of individuals with an IgM-only positive Serum-*Bb* (Serum-*Bb*-IgM), an IgG-only positive Serum-*Bb* (Serum-*Bb*-IgG) or an IgM and IgG positive Serum-*Bb* (Serum-*Bb*-IgM/IgG) who had a corresponding positive *Bb*-AI, respectively.

**Results** 36 617 individuals had a *Bb*-AI with a corresponding Serum-*Bb*. The proportion of individuals with a positive *Bb*-AI who had a corresponding Serum-*Bb*-neg was 16.4% (95% CI, 14.8-18.1). The proportion of individuals with a Serum-*Bb*-IgM, Serum-*Bb*-IgG or Serum-*Bb*-IgM/IgG who had a corresponding positive *Bb*-AI was 10.7% (95% CI, 9.7-11.8); 23.5% (95% CI, 22.0-25.1) or 43.0% (95% CI, 40.4-45.6), respectively. The proportion of individuals with Serum-*Bb*-IgM/IgG who had a corresponding positive *Bb*-AI was larger in individuals >18 years old (59.8% (95% CI, 53.6-65.8)).

**Conclusions** Analysis of *Bb* serum antibodies is of limited diagnostic value for LNB-diagnosis. Almost a fifth of those with a positive *Bb*-AI had Serum-*Bb*-neg and less than half with Serum-*Bb*-pos had a positive *Bb*-AI. More than a tenth of individuals with a positive *Bb*-AI had a negative corresponding Serum-*Bb*. In children more than half with Serum-*Bb*-IgM/IgG had positive *Bb*-AI.

**References**

Introduction
The recommended treatment length for early European Lyme neuroborreliosis (LNB) is 14 days according to EFNS guidelines from 2010 (1), as most published studies report treatment for 10 to 14 days, sometimes 28 days. In practice however, patients often receive prolonged courses of antibiotics (2). Systematic reviews (3, 4) from recent years found no evidence to suggest benefits of extended treatment, but both recommended further research. The main aim of this study was therefore to investigate whether 14 days treatment with oral doxycycline of European LNB in adults was as effective as a prolonged course for 6 weeks.

Material and method
The study had a multi-center, non-inferiority, randomized, placebo-controlled and double-blinded design. One hundred and twenty-one patients diagnosed with LNB according to EFNS guidelines were randomized to six or two weeks treatment with oral doxycycline. The primary endpoint was improvement on a composite clinical score (CCS) from baseline to six months after inclusion. Secondary endpoints were improvements in the CCS 12 months after inclusion, findings from patient reported questionnaires, and safety as measured by side effects of the two treatment arms. Blood and CSF were collected throughout the follow-up, and a biobank was established.

Results
One hundred and five patients were followed through to six months, and 94 patients were followed through to twelve months according to Intention to treat principle.

Discussion/Conclusion
We will present unpublished data and results from the study regarding the two treatment groups and long-term data.

References
O6. Regional blood-brain barrier function in Lyme neuroborreliosis patients: A dynamic contrast enhanced magnetic resonance imaging case-control study

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Introduction: Increase of protein, albumin and white blood cells in the cerebrospinal fluid (CSF) of patients with Lyme neuroborreliosis (LNB) indicate considerable nervous system inflammation and disturbed integrity of blood-brain barrier(1). The signal intensity-time curve from dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) together with application of pharmacokinetic models gives estimates for microvessel characteristics(2, 3). The study aim was to test if the transfer constant, a vessel permeability parameter, is different in LNB patients and controls, and if it is associated with CSF-serum albumin ratio.

Material and methods: 55 LNB patients and 15 controls were examined with 3D T1-weighted and DCE-MRI. Region of interest masks were auto-generated from the 3D T1 acquisition using FastSurfer(4). The DCE-MRI was acquired with 3D T1 spoiled gradient echo sequence with duration 9.6 minutes, contrast was injected intravenously after 10 frames. T1 mapping was performed prior to this in order to convert DCE signal intensity to contrast agent concentration. Transfer constant maps were generated based on the extended Tofts model. The patients had CSF analysis prior to MRI.

Results: The transfer constant in the temporal cortex and cerebellar white matter was significantly higher in patients compared to controls, and in the thalami, caudate nuclei and hippocampi it was significantly lower in patients compared to controls. There was a low-moderate degree of correlation between the transfer constant and serum-CSF albumin ratio in the caudate nuclei, parietal and temporal cortex.

Conclusion: This DCE-MRI study showed regional increases and reductions in brain microvessel permeability of LNB patients.

References:
O7. Cognitive function and fatigue in patients with acute neuroborreliosis

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Introduction

Long-term cognitive problems and fatigue after adequately treated neuroborreliosis has caused uncertainty and debate among patients and health care workers for years. Although complete recovery after treated neuroborreliosis is expected, some patients report persistent health problems like fatigue, myalgia, cognitive problems and reduced quality of life after treatment (1-4). Despite several studies, the prevalence, cause and severity of such complaints are still not clarified. More knowledge about cognitive function, fatigue and MRI findings in the acute phase of neuroborreliosis could possibly contribute to clarification. In the current study, we therefore aimed to address this.

Material and methods

Patients with well-characterized acute neuroborreliosis (n=72) and a matched control group (n=68) were screened with eight subtests from three different neuropsychological test batteries assessing attention, working memory and processing speed. Level of fatigue was measured with Fatigue Severity Scale, and Fazekas score was used to grade white matter hyperintensities on MRI.

Results

We found no significant differences in mean scores on the neuropsychological tests between the groups. The patient group reported significantly higher level of fatigue (Fatigue Severity Scale: 4.8 vs. 2.9, p<.001). There was no significant difference in Fazekas score between the groups.

Discussion/ conclusion

Neuroborreliosis does not seem to affect cognitive functions in the acute state of the disease, while fatigue is common.

References

An intervention in general practice to improve the management of Lyme borreliosis in Denmark

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Introduction/objectives
To improve the following outcomes in patients with Lyme borreliosis (LB) through an educational intervention in general practice; 1) increase number of referred patients on suspicion of LB, 2) increase number of lumbar punctures examined for Borrelia burgdorferi antibody index, 3) decrease number of Serum-B. burgdorferi antibody tests requisitioned, 4) shorten delay from symptom onset to treatment in Lyme neuroborreliosis (LNB) patients.

Material and methods
A prospective non-blinded non-randomized intervention trial in LNB high-incidence areas in Funen, Denmark. The intervention included education of general practitioners about ticks and LB, and was carried out 22.1.2019 – 7.5.2019. Results were compared between the intervention-group (49 practices) and the remaining general practices in Funen (71 practices) in the two years before and after the intervention.

Results
In the study period, 196 patients were referred on suspicion of LB, a 28.9% increase in the intervention-group post-intervention and a 59.5% increase in the control-group (p=0.47). The number of CSF-Borrelia-Antibody Index tests increased 20.8% in the intervention-group and 18.0% in the control-group (p=0.68). 3.5% had intrathecal antibody-production, more frequent in the intervention-group (5.0%) compared with the control-group (2.5%, p=0.005), but with declining prevalence after intervention. The number of requisitioned Serum-B. burgdorferi antibody tests declined 43.1% in the intervention-group, 34.5% in the control-group (p=0.30). 25.1% had presence of Serum-B. burgdorferi antibodies. We found no difference in LNB treatment delay before and after intervention or between groups (p=0.21).

Conclusions
We found an overall improvement in LB awareness and referrals among general practitioners, but could not show any effect of the intervention on treatment delay or risk of LNB sequelae.
09. *Candidatus* Neoehrlichia mikurensis in Danish immunosuppressed hematological patients: a retrospective cohort study

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**Introduction**

*Candidatus* (Ca.) Neoehrlichia mikurensis is an emerging tick-borne bacterium which can cause the disease, neoehrlichiosis(1). More severe disease courses have been seen in immunocompromised patients including thromboembolic complications(2). In Denmark, only one published case of neoehrlichiosis has been described(3). Screening for Ca. Neoehrlichia mikurensis after a tick-bite is not routine in Denmark. Therefore, we hypothesized that Ca. Neoehrlichia mikurensis is underestimated in Danish patients receiving biological therapy.

**Methods**

In the PERSIMUNE biobank, we localized 239 unique plasma samples from hematological and rheumatological patients treated with either anti-CD20 antibodies or TNF-α, collected between 2015-2019. A real-time PCR targeting the groEL gene was used to detect Ca. Neoehrlichia mikurensis DNA. Positive real-time PCR amplicons were further amplified by PCR and sequenced for confirmation.

**Results**

In total Ca. Neoehrlichia mikurensis DNA was detected in 3 of the 239 plasma samples. The patients (Female/Male: 2/1) were all treated with anti-CD20 antibodies; One had idiopathic thrombocytopenia, and two chronic lymphatic leukemia.

**Conclusion**

Neoehrlichiosis is likely overlooked in Danish immunosuppressed patients, however further investigations regarding the extent consequences are yet to be investigated.

3. Porskrog A, Himmelstrup BM. [Tick-borne CandidatusNeoehrlichiamikurensis was the cause of fever in a haematological patient]. Ugeskrift for laeger. 2020;182(40
O10. The consequences of trophic resources and habitat variability on rodent host and tick burden

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Introduction:
Emergence of tick-borne diseases is related to host-pathogen-parasite system alterations, which are affected by several factors. In this sense, underlining the mechanisms between environmental heterogeneity, hosts availability and tick burden are of particular interest to understand the circulation of tick-borne pathogens.

Materials & Methods:
The study was carried out in the north-eastern Italian Alps from February 2019 to April 2021. Wild rodents were capture-mark-recaptured in three control and three treatment grids where ad libitum supplemental feeding was provided. Spatial-Explicit-Capture-Recapture Models and Generalized Additive Models were applied to evaluate tick burden in relation with vegetation cover at ground level, rodent density and contrasting food resources.

Results:
390 Apodemus flavicollis and 109 Myodes glareolus were captured and 1760 feeding ticks of different Ixodes ricinus stages were counted. The additional food positively influenced A. flavicollis by increasing its density and shrinking homeranges, which in turn decreased M. glareolus ones. Additionally, rodent density and environmental structure diversely contributed in defining tick burden especially in A. flavicollis rather than in M. glareolus.

Conclusion:
Our findings support the complex relationship that link tick burden to rodent hosts. Indeed, at local scale, habitat heterogeneity and availability of trophic resources affect rodent density and space use pattern, contributing to the identification of potential hotspots for disease transmission.

References:
Variation in tick density and pathogen prevalence between greenspaces has been well documented. In contrast, variation within greenspaces remains poorly understood, although vital for understanding local disease risk. We captured ticks by drag-sampling in 2018, 2019 and 2020 at 36 locations in 10 greenspaces within the province of Antwerp, Belgium. Each location contained three location types, characterised by greenspace infrastructure and associated levels of human activity: (1) recreational infrastructure (e.g. bench); (2) 40 meters further along the connecting trail and; (3) the interior of the associated forest stand, at equal distance from (1) and (2). Captured nymphs and adults were tested for a selection of pathogens, including *Borrelia burgdorferi* s.l.. Density of questing nymphs (DON) was highest in the forest interior and considerably lower along trails and benches. Ticks were also more abundant in structure-rich compared to structure-poor stands and in deciduous compared to coniferous stands. This enables the prediction of DON near infrastructure, compared to other stands within the same greenspace, based on forest stand characteristics (Van Gestel et al., 2021). A preliminary analysis of prevalence data suggests spatial sorting in *Borrelia* prevalence on a micro-geographic scale, with higher nymphal infection prevalence along trails and infrastructure compared to forest interior. This might be explained by the habitat use of hosts, as incompetent hosts spend more time in the forest interior, as was observed by the deployment of camera traps at each location type.

Cited reference:
O12. Serological analysis of Tick-borne encephalitis virus in Norway

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Tick-borne encephalitis virus (TBEV) is widespread with increased incidence and prevalence in Europe over the last decades. TBEV is an arbovirus and a member of the Flaviviridae family that are known to cross-react in various analytical assays. Due to this cross-reactivity there is a ongoing need to design and validate new assays with improved specificity and sensitivity for detection of different flaviviruses in human and animal serum.

The Norwegian Institute of Public Health (NIPH) has since 2008 been responsible for the national reference function (NRF) for Tick-borne encephalitis (TBE). Improvement and replacement of assays is part of the mandate for NRF. Due to the discontinued production of Enzygnost anti-TBE/FSME/ETG-virus (IgG, IgM) ELISA from Siemens/DiaZorin last year, NIPH had to perform a validation of several available TBE assays for replacement of the test. To complement the serology assays for distinguishing TBEV vaccinated from previously infected TBEV patients we included a TBE NS1 ELISA.

We have tested four commercial and one non-commercial ELISAs targeting the TBEV Envelope- and the NS1 protein, respectively. In addition, we have analysed human serum samples by Enzygnost anti-TBE/FSME/ETG-virus ELISA on the automatic DS2 DYNEX analyser. The same kit was used for manual sheep serum analysis. A selection of serum samples from both assays were confirmed by virus neutralization test.

There is a broad variation in both sensitivity and specificity of the different ELISA methods. It is a challenge to find an assay that have high specificity for TBE detection of both human and animal samples.
P1. Studying the role of *Borrelia bavariensis* PFam54 complement-interacting proteins in vitro and in vivo using two strains naturally lacking the PFam54 gene array

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Lyme borreliosis is the most common vector-borne disease in the Northern hemisphere, caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato (Bbsl) species complex which are transmitted by ixodid ticks [1, 2]. Bbsl species produce a family of proteins (PFam54) encoded by genes located on the linear plasmid 54, some of which confer the functions of cell adhesion and inactivation of complement, the first line of host defense [3–5]. However, the impact of PFam54 in promoting Bbsl pathogenesis remains unclear because of the hurdles to simultaneously knock out all PFam54 encoding genes in a spirochete. Here, we found two *Borrelia bavariensis* (Bbav) strains isolated from patients, PBN and PNi, naturally lacking PFam54 but maintaining the rest of the genome with greater than 95% identity to the reference Bbav isolate PBi. We found that PBN and PNi less efficiently survive in human serum than PBi. Such defects were restored by introducing two Bbav PFam54 recombinant proteins, BGA66 and BGA71, confirming the role of these proteins in providing complement evasion of Bbav. Further, we found that all three strains remain detectable in heart tissue 21 days post subcutaneous mouse infection, supporting the non-essential role of Bbav PFam54 in promoting spirochete persistence although potential differences in tissue tropism between the three strains were observed. This study identified and utilized isolates deficient in PFam54 to associate the defects with the absence of these proteins, building the foundation to further study the role of each PFam54 protein in contributing to Bbsl pathogenesis.

References


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Introduction
Lyme borreliosis (LB) is the most common tick-borne disease in Germany [1]. Estimates of prevalence of infections with Borrelia (B.) burgdorferi and LB are incomprehensive [2-5].

Material and methods
Based on two cross-sectional surveys, we estimated the B. burgdorferi-specific antibody seroprevalence, representative of 3–17-year-olds in Germany. Using sample pairs, we estimated yearly seroconversion and seroreversion rates. We assessed risk factors for seropositivity, seroconversion and seroreversion.

Results
Seroprevalence was 4.4% (95%CI: 3.9–4.9) in 2003–2006 and 4.1% (95%CI: 3.9–4.9) in 2014–2017. It increased from 2.3% (95%CI: 1.6–3.1) in 2003-2006 and 1.4% (95%CI: 0.2–2.5) in 2014–2017 in 3–6-year-olds to >6% (95%CI: 4.6–9.1) in 14–17 year-olds in 2003–2006 and 2014–2017. Seroprevalence was significantly higher in boys, in the southeastern regions of Germany, in participants with high socio-economic status, and, in the first survey, in participants residing in rural areas. Boys compared to girls, minors vs. young adults, and children residing in rural areas had higher odds of seroconversion. Low antibody levels were the main predictor of seroreversion. The yearly seroconversion rate was 0.3%, the yearly seroreversion rate 3.9%.

Conclusion
Seroprevalence was stable between 2003–2006 and 2014–2017. Most predictors of seropositivity remained the same. Seroconversion was more likely in minors than in young adults, indicating high exposure at younger age. Frequent infections among minors justify targeted prevention efforts.

P3. Blackbirds a key reservoir of neuroinvasive strains of *Borrelia garinii*

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*Borrelia burgdorferi* sensu lato (Bbsl) currently represents a complex of 22 genospecies (1, 2). *Borrelia garinii*, which is associated with avian reservoirs, is one of the most genetically diverse and widespread human pathogenic genospecies from the Bbsl complex.

Ticks and birds were sampled in south-east Slovakia. Sampled birds were examined for feeding ticks and questing ticks were collected at the same study site. Ticks were collected during spring and fall migration and nesting season (2017–2019).

In total, 1330 birds belonging to 56 species were examined. 1652 *I. ricinus* ticks were collected from 20 species of birds. 9 bird species carried *Borrelia* infected ticks and 44.4% of collected ticks were infected. Blackbirds had the highest reservoir capacity whereas fed 82.7% infected ticks. *B. garinii* and *B. valaisiana* predominated, representing more than 97% of all bird associated Borrelial genospecies. 184 of 672 (27.4%) questing ticks carried Bbsl that was represented by five genospecies. To better understand the importance of birds on the dispersion of Borrelia, *B. garinii* from bird feeding and questing ticks were further analysed by multilocus sequence typing (MLST). MLST analysis of concatenated sequences revealed its high intraspecific variability. Four unique sequence types were described. Moreover *B. garinii* ST, that were isolated from patients with neuroborreliosis, were detected in bird feeding ticks. Using MLST we have proved the importance of blackbirds as an important reservoir hosts of neuroinvasive strains of *Borrelia garinii*.

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References.

P4. The lack of the association of the CCR5 genotype with the risk and clinical presentation of tick-borne encephalitis

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Introduction
The host factors influencing the susceptibility to and the severity of tick-borne encephalitis (TBE) are poorly defined. Chemokines and their receptors shape the immune response to neuroinfection by driving leukocyte migration into central nervous system and cerebrospinal fluid (CSF). The loss-of-function ∆32 mutation in the chemokine receptor gene CCR5 is a risk factor for West Nile encephalitis and was found to associate with the risk and clinical severity of TBE in the Lithuanian population, suggesting a protective role of CCR5 in Flavivirus encephalitis.

Material and methods
We have studied CCR5 genotype in 205 TBE patients stratified by clinical presentation and 260 controls from the same area (Podlasie, Poland). The genotype distribution between groups and subgroups was compared with chi-square test and quantitative differences between genotypes with Mann-Whitney test, with p<0.05 considered significant.

Results
There were 36 (17.6%) CCR5 ∆32 heterozygotes and 3 (1.5%) homozygotes in TBE group, with no difference in comparison with the control group. The CCR5∆32 allele did not associate with the clinical presentation of TBE (menigitis, meningoencephalitis or meningoencephalomyelitis), its severity, decreased consciousness level, paresis or cerebellar syndrome. The CSF inflammatory parameters did not differ between the wild type (wt/wt) and wt/CCR5∆32 genotype. The clinical presentation in all three ∆32/∆32 homozygotes was mild and their CSF parameters unremarkable.

Conclusions
The CCR5∆32 mutation did not correlate with the risk and presentation of TBE, which challenges the widely assumed protective role of CCR5 in TBE. CCR5 is not indispensable for the effective immune response against TBE virus.

References
Early European Lyme neuroborreliosis - comparison of different clinical manifestations

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Background. Information on the relative frequency, and the course and outcome of different clinical manifestations of early European Lyme neuroborreliosis (LNB) is limited.

Methods. This prospective study comprised adult patients, diagnosed with early LNB at a single centre in 2005–2019. The diagnosis of proven LNB was based on: i) cerebrospinal fluid (CSF) pleocytosis and ii) intrathecal synthesis of borrelial antibodies and/or positive CSF borrelial culture and/or the presence of erythema migrans (EM). Three major clinical entities (meningoradiculoneuritis (Bannwarth syndrome (BS)), peripheral facial palsy (PFP) and EM-associated meningitis without radicular pain) were assessed and compared.

Results. 276/1302 patients fulfilled criteria for proven LNB: 165/254 (65%) with clinically suspected BS, 40/208 (19%) with EM associated with “neurologic” symptoms but without radicular pain, and 71/840 (8%) who presented with cranial neuropathy. Compared to the other two groups, patients with BS were older (60 vs. 50 years), had longer duration of symptoms (26 vs. 16 days), larger EM diameter (30 vs. 15 cm), higher CSF leukocyte count (138 vs. 62×10^6/L) and protein concentration (1.2 vs. 0.7 g/L), and higher borrelia isolation rate from skin (36.4% vs. 21.9%) with marked predominance of Borrelia garinii isolates (22/23, 95.7% vs. 2/7, 28.6%). Outcome was favourable (93.7% of patients were symptom free or had marked regression of symptoms/signs 12 months after 14-day ceftriaxone or doxycycline treatment) and was comparable in the three groups.

Conclusions. BS is the most frequent manifestation of early European LNB. It distinguishes from other early LNB manifestations in symptomology, laboratory findings, and etiology.
P6. Illness Caused by Tick-Borne Encephalitis Virus without Central Nervous System Involvement: Clinical and Laboratory Characteristics and Outcome

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Introduction. Information on febrile illness caused by tick-borne encephalitis virus (TBEV), but without central nervous system involvement, is limited.

Methods. We characterized in detail 98 such patients with TBEV RNA in their blood.

Results. The median duration of illness was 7 days; 37/98 (38%) patients were hospitalized. The most frequent findings were malaise/fatigue (98%), fever (97%), headache (86%), and myalgias (54%), together with leukopenia (88%), thrombocytopenia (59%), and abnormal liver test results (63%). During the illness, blood leukocyte counts tended to improve, whereas thrombocytopenia and liver enzymes tended to deteriorate. At the time of positive PCR findings, 0/98 and 7/98 patients had serum IgG or IgM TBEV antibodies, respectively; all the patients later seroconverted. The viral RNA load was higher in patients with more severe illness, but did not differ substantially in relation to several other factors. The illness progressed to TBE in 84% of patients within 18 days after defervescence.

Conclusions. The illness caused by TBEV, but without central nervous system involvement, may be a quite severe. Clinical and laboratory findings in patients with TBEV febrile illness do not distinguish between patients who later develop TBE and those who do not.
P7 Predictors of individual performance and evolutionary potential of life-history traits in a bird-specialized tick

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Understanding how tick traits covary, evolve, and how they contribute to tick fitness is fundamental to comprehend how ticks adapt and evolve. Nevertheless, within- and between-individual variation of tick life-history traits and their contribution to tick fitness are poorly understood. We investigated how life-history traits predict success of individual tree-hole ticks Ixodes arboricola and estimated their evolutionary potential, as well as genetic correlations within stages and phenotypic correlations within and across stages. We raised a lab population of ticks that exclusively fed on great tits Parus major and followed them individually over two generations. Feeding time, engorgement weight, moulting time, and number of hatched eggs were measured. Short feeding times and high engorgement weights strongly increased moulting success after accounting for tick maternal and host effects. Moreover, moulting time was positively correlated with feeding success in adults. In larvae and nymphs we found negative phenotypic correlations between engorgement weight and both feeding and moulting time, the latter supported by a negative genetic correlation. Differences between males and females were found in feeding time (longer in male nymphs) and moulting time (longer in male larvae, shorter in male nymphs). Time since the last feeding event (set experimentally) reduced fitness of larvae and nymphs while it increased adult female fitness. We hypothesize that variation in tick fitness is shaped by consistent individual differences in tick quality rather than by life-history trade-offs. Furthermore, we found significant heritability and evolvability (potential to respond to selection) for engorgement weight and moulting time across all stages.
P8. *Borrelia* infections in ageing field collected *Ixodes ricinus* ticks

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In Europe, *Ixodes ricinus* plays a major role as a vector of *Borrelia burgdorferi* sensu lato (s.l.) spirochaetes, the causative agents of Lyme borreliosis, among other pathogens. In unfed ticks, extracellular pathogens such as *Borrelia* spirochaetes experience prolonged nutrient restriction. However, only few studies exist with regard to *Borrelia* infections in unfed ticks of different physiological age. As unfed ticks consume their energy reserves over time, their body dimensions change. Thus, measurement of the soft alloscutum in relation to the invariable scutum can be used to derive the so-called morphometric age ratio, which allows an estimate of the physiological age of unfed ticks [1]. The present study investigated the relationship of morphometric age with *Borrelia* infection status and spirochaete load in 1,882 questing *I. ricinus* nymphs, collected from March to October 2020 at two different locations in northern Germany. In addition, *Borrelia* species composition was investigated by employing a reverse line blot (RLB) probe panel suitable for the detection of ten different *B. burgdorferi* s.l. species, as well as *B. miyamotoi*. Overall *Borrelia* prevalence was 25.8% (485/1,882). Whilst there was no statistically significant difference in *Borrelia* prevalence between the different morphometric age groups, *Borrelia* copy numbers as determined by probe-based quantitative real-time PCR significantly declined with increasing morphometric age. *Borrelia* species differentiation by RLB was successful in 18.8% of positive ticks, and revealed *B. afzelii* as the dominating species (72.5% of differentiated infections). In addition, *B. garinii, B. valaisiana, B. burgdorferi* sensu stricto, *B. spielmanii* and *B. miyamotoi* were detected.

References:
Prevalence and genetic variability of *Borrelia burgdorferi* sensu lato is linked to different ecological aspects such as habitats in different areas and the resident reservoir hosts. Therefore, we have chosen five different European localities to find out if and how the prevalence and population structure of *B. burgdorferi* s.l. is affected by the geographical latitude.

Questing ticks were collected on study sites in Slovakia, Ukraine, Croatia, Bulgaria and Finland in the years 2011 – 2019 and screened for the presence of *B. burgdorferi* s.l. by amplifying the 5S-23S rRNA intergenic spacer (Derdakova et al. 2003). 97 samples were further tested using Multilocus Sequence Typing (MLST, Margos et al. 2008) and the concatenated sequences of the eight MLST loci were analyzed using Maximum Likelihood (MEGA 7) and goeBurst (Phyloviz). Additional samples were downloaded from the MLST database (pubmlst.org) for comparison.

The highest *B. burgdorferi* s.l. prevalence was detected in Slovakia (26.7 %, 282/1058), followed by Croatia (23.5 %, 212/901), Ukraine (22 %, 263/1195), Finland (12.4 %, 94/760) and Bulgaria (6.8 %, 18/265). The highest interspecific variability was recorded in Ukraine and Croatia/Slovakia, with the presence of seven and six genospecies, respectively. In Finland, only three genospecies were found. Out of 97 samples, 63 (65 %) were defined as new sequence types (ST). The highest variability was observed in Ukraine with 12 STs (80 %) detected in 15 samples. Contrarily, in Finland only three new STs (60 %) were found.

According to the phylogenetic tree, *B. lusitaniae* isolates from Croatia, Bulgaria, and Slovakia fell into Central/Eastern European group of this species. *B. bavariensis* samples from Ukraine clustered together with European isolates, separate from the *B. bavariensis* strains from Asia. *B. burgdorferi* s.s. samples from Slovakia and Ukraine were homogeneous.

In conclusion, our findings indicate the lowest prevalence and genetic variability of *B. burgdorferi* s.l. in higher geographical latitudes.

References:
Background. In Europe, Lyme neuroborreliosis (LNB) is diagnosed based on the finding of intrathecally produced Borrelia-specific IgM and/or IgG antibodies. We aimed to examine if the time from symptom debut to lumbar puncture (LP) affected findings of intrathecal production of Borrelia-specific IgM and/or IgG antibodies in patients with LNB.

Methods. A retrospective study of 544 patients with a positive Borrelia Burgdorferi antibody index (Bb-AI), analysed at the Department of Clinical Microbiology at Odense University Hospital (OUH), Denmark, between 01.01.1995 and 31.12.2020.

Results. The delay from symptom onset to LP was for patients with positive Bb-AI IgM 30 days (IQR 14 – 95 days), IgG 24 days (IQR 11 – 62 days) and IgM+IgG 24 days (IQR 14 – 48 days), P=0.098. Ninety-three patients had a second, and 25 had a third LP performed after a median of 125 (IQR 28 – 432 days) and 282 days (IQR 64 – 539 days), respectively. The majority of patients (66.7%) did not convert from their initial intrathecal antibody finding. Patients with isolated positive Bb-AI IgG had a higher prevalence of cognitive symptoms (9.0%) compared to patients with isolated positive IgM (2.9%, P=0.012) and of sensibility disturbances (12.3%) compared to patients with isolated positive IgM (1.7%, P<0.001) or IgM+IgG (6.1%, P=0.041).

Conclusions. The intrathecal Borrelia-specific antibody production does not follow the otherwise well-known immune response with an initial IgM followed by an IgG production. Based on our results, physicians diagnosing LNB patients should not base their interpretation of the stage of the disease on the type of antibodies found in the cerebrospinal fluid.

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Introduction:
Diagnosis of Lyme disease, in the early stages of the infection, is very complex. Over the course of several days to a few weeks after initial infection, antibody response against B. burgdorferi is not detectable. The conventional serologic testing is a standard two-tier testing (sTTT) employing an enzyme or chemiluminescence immunoassays in the first tier, followed by confirmatory immunoblotting in the second tier for positive or equivocal results in which sensitivity rarely exceeds 50% and specificity 95%.

LIAISON® LymeDetect® is a new solution for early diagnosis of Lyme borreliosis. It combines current standard serology testing (IgG/IgM antibodies detection) with an interferon-gamma release assay specific to Borrelia burgdorferi. Interferon-gamma responses to B. burgdorferi antigens seem to be capable of detecting the infection earlier than antibody tests.

Material and methods:
A prospective study enrolling over 300 subjects (patients in the early stages of the infection and healthy donors) was performed in Poland, Czech Republic, France, Germany, and Italy.

IgG and IgM testing was done in serum, while blood collected in lithium heparin tubes was added to Nil, Mitogen and Lyme-specific tubes containing peptides to Borrelia burgdorferi for subsequent measurement of interferon-gamma. Tests were performed on the LIAISON® XL automated chemiluminescence system. Results were combined into a single qualitative patient report (positive, negative or indeterminate) to assess the early diagnosis.

Results:
309 samples results were eligible for the analysis. Diagnostic sensitivity and specificity were calculated for LIAISON® LymeDetect® and the diagnostics standard of care, standard Two-Tier Testing (sTTT).

Diagnostic sensitivity:
- From 3 to 6 weeks: 84.6% LIAISON® LymeDetect® vs 61.5% sTTT;
- After more than 6 weeks: 81.0% LIAISON® LymeDetect® vs 69.0% sTTT.
- Diagnostic specificity resulted 100.0% for both sTTT and LIAISON® LymeDetect®.

Discussion/Conclusions:
From current data, LIAISON® LymeDetect® provides a higher gain on sensitivity in the early phases of the infection (≤3 and 3-6 weeks) compared to the current sTTT. A recent publication supports also the potential use of interferon-gamma detection at varying stages of Lyme disease: additional studies are being performed.

Bibliography
Prevalence of *Borrelia afzelii* in ticks from the ‘West-Norwegian Neoehrlichia gap’

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**Introduction.** *Neoehrlichia mikurensis* is widespread in Norway and in many places, the prevalence is as high as in anywhere else in the world (1). *N. mikurensis* is abundant both in the Southeast and in the North. However, along the more southerly parts of the western coast it is virtually absent (2). *N. mikurensis* is associated with *Borrelia afzelii* and coinfected ticks are more than 2x more common than expected by chance. It is thus natural to ask whether the low *N. mikurensis* prevalence on the western seaboard of Norway is paralleled by a correspondingly low *B. afzelii* prevalence.

**Materials and methods.** Ticks from a previous study of *N. mikurensis* along the Norwegian coast (2) were tested for *B. afzelii* by species-specific real time PCR based on an earlier conventional PCR targeting the ospA gene.

**Results.** Preliminary results from ongoing experiments indicate that low prevalence of *N. mikurensis* is associated with low prevalence of *B. afzelii*.

**Discussion.** The results are consistent with *N. mikurensis* and *B. afzelii* having a common reservoir host that does not thrive in the mild and wet conditions of coastal southwestern Norway.

**References.**