**COST STSM TD1303-24845 report**

**COST Action TD1303**

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**Host institution:** James H. Oliver Jr. Institute for Coastal Plain Sciences

**Hosts:** Dr. Daniel Gleason

**Reference code**: COST-STSM-ECOST-STSM-TD1303-180215-057872

**STSM Reference number**: TD1303-24845

**Period:** 24/05/2015 to 16/06/2015

**STSM title:** Transoceanic transmission of *Borrelia garinii,* the causative agent of Lyme disease, from Europe to the southeastern United States: analysis of co-infected isolates.

**Amount approved**: EUR 2000

## STSM objective

The main objective of the conducted STSM was to confirm the presence of "European" species *B. garinii* in the southeastern United States as a result of transoceanic migration of a variety of migratory animal species, particularly birds from Europe. Our preliminary results suggest that the representation of *Borrelia* species pathogenic to humans in the New World is larger than is reported and the spirochete distribution is supported by a broad expansion of infected ticks by migratory hosts.

## Methods

Collection of the southeastern US *Borrelia* isolates was started in 1991 by Prof. J. H. Oliver Jr. Today the collection is housed in the James H. Oliver Jr. Institute for Coastal Plain Sciences (Statesboro, Georgia). It consists of more than 300 cultured isolates from 6 hard tick species, 3 rodent host species, 8 bird species and extensive group of isolates, originated from different sources, with the confirmed presence of multiple spirochete species. We have an open access to this collection, approved by its owner and the Director of the ICPS. At this time we directed our attention to the group of unanalyzed samples originated from the coastal area of the southeastern Unites States from which 25 isolates were selected for our detailed analysis.

First of all, to secure the permanent availability of the samples, isolates involved in this STSM were re-cultivated by scraping the frozen stocks and incubating spirochetes at 33$^{o}$C in BSK-H complete media for 3 weeks (until departure). Control of spirochete multiplication was done using dark field microscope once a week. Total *Borrelia* DNA was purified from aliquots of frozen stocks using DNeasy Blood and Tissue kit (Qiagen, U.S.A.). The MasterTaq Kit (Eppendorf, Germany) that contains recombinant Taq DNA polymerase from *E. coli* DH1 and a special 5×TaqMaster PCR enhancer was used for amplification of spirochete 5S-23S intergenic region using *B. garinii* specific primers as well as gene encoding *flagellin* using *B. burgdorferi* sensu lato specific primers. The special dNTPs mixture was used in PCR reaction due to the high A/T content in *Borrelia* genome. It was prepared by mixing of 10mM dNTPs in ratio 26A:26T:14C:14G, and is used in a final concentration of

0.2mM. Amplifications were conducted in the 25 µl in 0.2 ml thin wall PCR tubes according to Eppendorf protocol. Mastercycler® ep system with 96 wells thermo block and heated lid technology was used. The PCR conditions for the amplification of the 5S-23S ITG and *fla* gene were as follows: initial denaturation at 96°C for 5 min, followed by 30 cycles at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min and the final extension step at 72°C for 10 min. Reactions were set up in a separate area with all precautions (supplies, equipment, and employee's personal safety items, pre- and postamplification activities). A negative control (no template) and positive control (*B. burgdorferi* B31 DNA as a template) were added to each amplification reaction. All PCR products were separated by 2% agarose gel, cut off the gel and purified using MinElute Gel Extraction kit (Qiagen, U.S.A.). Purified PCR products were submitted for direct sequencing to the University of Washington High-Throughput Genomic Unit (Seattle, USA). Sequences were conducted in both directions, using the same specific primers that were used for amplification of each gene. All sequences were analyzed with EditSeq module of DNASTAR software (DNASTAR, United Kingdom). Database searches used the BLAST programs of the NCBI (Bethesda, USA).

**Results**

Out of 25 selected isolates collected from rodent and bird hosts from coastal area of South Carolina and Georgia (USA), **five** (**5**!) produced the *B. garinii*-specific PCR product in amplification with 5S-23S ITG primers and total DNA as a template. The presence of *B. garinii* as co-infected species was conformed in three isolates (SCCH-7, SCGT-19 and SCI-8) from South Carolina and 2 isolates (BUL-12 and GAC-6) from Georgia.

The exact sizes of the 5S-23S intergenic spacer regions (253 nucleotides) of the tested *Borrelia* isolates were determined by direct sequencing of the purified amplicons. The intergenic spacer sequences of two isolates, SCCH-7 and SCGT-19, were 100% identical between themselves and exhibited the highest (97%) sequence similarity in this locus with *B. garinii* 20047 isolate from China (KJ459339). The *in silico* digestion with DraI and MseI restriction enzymes revealed the fragments of 201 bp and 52 bp, and 107 bp, 95 bp and 51 bp respectively, confirming the RFLP patterns specific for *B. garinii*.

Amplicons of the *flagellin* gene (497 nt) from SCCH-7 and SCGT-19 isolates were 99% similar between themselves and revealed 99% and 98% of similarity in this locus to different *B. garinii* isolates and strains. The *in silico* digestion of adjusted 488 nt long fragment of *flagellin* sequence with CelII, DdeI, HapII, HhaI and HincII restriction enzymes revealed RFLP pattern characteristic for *B. garinii* in both isolates, i.e. the absence of restriction sites for CelII and HapII restriction endonucleases, 329 bp, 78 bp, 72 bp, and 9 bp long fragments in case of DdeI digestion, 405 bp and 83 bp long fragments in case of HhaI digestion and 453 bp and 35 bp long fragments in case of HincII digestion.

**Plans**

Analysis of amplified sequences from isolates SCI-8, BUL-12 and GAC-6 is in progress. Developed cultures of co-infected spirochete isolates involved in this STSM were frozen and sent to Biology Centre, Institute of Parasitology in the Czech Republic. To isolate the monoclonal culture of *B. garinii* from co-infected southeastern US samples, all isolates will be plated into solid BSK-II media. The developed individual colonies will be re-cultivated in liquid BSK-II media and confirmation of identity of monoclonal cultures to *B. garinii* will be done by multilocus sequence analysis or multilocus sequence typing methods that we use routinely in our laboratory. Obtaining of isolated monoclonal culture of "European" species in southeastern US samples will confirm our theory regarding the wide distribution of spirochetes by migratory hosts. It is interesting to note that out of 5 samples that showed the presence of *B. garinii* in them 4 were isolated from rodent hosts characteristic

to southeastern USA,- *Peromyscus gossypinus*, *Sigmodon hispidus* and *Neotoma floridana* (SCCH-7 and GAC-6, BUL-12, SCGT-19, respectively). And only one isolate, SCI-8, was bird-originated. All isolates were collected 20 years ago, in 1995, that supports the fact that *B. garinii* represents a well established spirochete population in the southeastern United States and might be another cause of Lyme disease in North America.

**Collaboration**

Upon completion, results of this STSM will be published in international peer-reviewed journal and the joint grant proposal involving Georgia Southern University (Statesboro, USA) and Biology Centre (Ceske Budejovice, Czech Republic) will be developed and submitted not later than in 2016.

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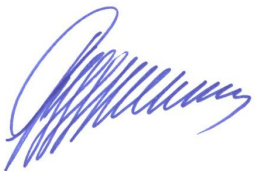

**Confirmation by the host institution of the successful execution of the STSM**

It is my pleasure to confirm that STSM of Dr. Rudenko Nataliia, PhD from Biology Centre, Institute of Parasitology in Georgia Southern University, James H. Oliver Jr. Institute for Costal Plain Sciences (Statesboro, USA) was successful. I herein confirm the present report regarding the COST-STSM-ECOST-STSM-TD1303-180215-057872.

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