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STSM SCIENTIFIC REPORT

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COST Action: TD1303

STSM Title: "Molecular diagnosis and phylogeny of *Bartonella* spp."

WG 5: Rare and emerging vector-borne pathogens

Reference: COST-STSM-ECOST-STSM-TD1303-080914-048135

STSM Period: 08/09/2014 to 25/09/2014

Location: KIT Biomedical Research, Amsterdam, The Netherlands

Host: Anna Paziewska-Harris,KIT Biomedical Research,Amsterdam(NL), A.Paziewska@kit.nl

Purpose of the STSM

The aim of this STSM was to learn how to analyse molecular data obtained during first two years of my PhD project using different approaches and to learn how to further interpret obtained results.

Description of the work carried out during the STSM

The work was done at the Host institution and it followed previously proposed work plan. During the whole duration of STSM, I had a chance to attend scientific meetings of Parasitological group at KIT Biomedical Research and to discuss with them different methods of pathogen detection in the area of infectious diseases. At the same time, I had a great opportunity to present my current work and to discuss my project with different scientists, which resulted in gaining valuable suggestions and advices.

Since my PhD project aims at detection, diagnostics and analysis of diversity and evolutionary relationships between zoonotic pathogens in rodents and their ectoparasites (fleas and ticks), during the first week of my stay, we have discussed essential issues related to laboratory work, such as optimising PCR conditions and possibilities of designing specific primers to reliably amplify pathogens from their hosts and vectors. This is of crucial importance for obtaining valid molecular results on a long-term basis. I have also learnt how

to correctly process obtained electropherograms of 16S-23S intergenic spacer region and of two housekeeping genes (*glt*A and *rpo*B) of *Bartonella* spp. isolates from rodents and how to build a proper sequence alignment using Clustal W and Muscle. We discussed pros and cons of using multi locus sequence typing approach and differences between the type of information that is generated by sequencing of non-coding fragments of DNA and housekeeping genes. In the 2nd week of this internship, I gained skills in drawing phylogenetic trees using MEGA software and I got accustomed with the criteria which help choosing between different methods of calculating trees the one that is most suitable for particular type of data. We also discussed the purpose of finding the best substitution model for DNA analysis and I became acquainted with calculating the distance matrix from the multiple sequence alignment. Later on, I have been introduced to the RDP2 program used for detection of recombination events within genes and to nested clade analysis approach (TCS software), which can be applied to identify clonal population structure as well as recombination events within highly divergent genera based on different genomic markers. I learned to work with Swiss model program which is used in protein structure modelling and with Mfold software for nucleic acid (RNA, DNA) folding and hybridization prediction.

Based on obtained results from cladograms and phylogenetic trees, during the 3rd week of my stay, we looked at some ecological and epidemiological associations between *Bartonella* sp. and rodents from my study system. We agreed on the next steps which need to be taken in my project to complete the picture of *Bartonella* epidemiology in my study system.

Description of the main results obtained

As mentioned above, during this short mission I had the opportunity to deepen my current knowledge in analysing molecular data using different phylogenetic techniques and I became familiar with methods for detection of bacterial recombination.

Within previously collected rodent samples, we have observed great genomic divergence of *Bartonella* sp., often with multiple infections present in one host. Analysis of 16S-23S ITS sequences from 44 isolates indicated higher prevalence of *Bartonella taylorii*, which could be divided into the three main clades. One clade of each, *B. grahamii* and *B. elizabethae* was also present. 12 isolates formed a separate clade which could not be assigned to any known species so far. Nested clade analysis of *gltA* and *rpoB* gene fragments showed host specificity of bacteria on the level of 1st step. Three isolates of *Bartonella* from *Apodemus agrarius* did not group with any of clades. Therefore, the short-term goals are as follows:

1. To obtain the adequate number of sequences of *gltA* and *rpoB* genes from *Bartonella* isolated from rodents according to the rodent species from different model sites within my study system, for comparative analysis.
2. To expand analysis of *Bartonella* from rodents to other housekeeping genes (*groEL, ribC* and *ftsZ*) to enhance the chance of detection of possible recombination events.
3. To investigate the diversity of *Bartonella* infecting *A. agrarius* in greater depth. Recently, Hildebrand *et al.* 2013 proposed that unusual range of *Bartonella* spp. is able to infect *A. agrarius*, however further analysis is needed to reveal the exact role of these bartonellae in diversity and recombination events within the genus. Therefore, as it seems a relevant issue for *Bartonella* epidemiology in my study system, my research will be focused on diversity of *Bartonella* spp. in *A. agrarius*.

Since fleas are known to be vectors of this pathogen, it is so important to understand their role in *Bartonella* evolution. Their ability to change hosts frequently during their lifetime, gives the opportunity for horizontal gene transfer between different strains of bacteria they transmit, which may result in the emergence of new genotypes. Therefore, the long-term aims to complete my study will be to genotype *Bartonella* detected in fleas and to compare strains obtained from rodents with those from fleas. This will allow answering general questions about molecular divergence of these bacteria and will enable to draw a broader picture of *Bartonella* epidemiology and identify possible implications to human health.

Future collaboration with the host institution (if applicable)

The present STSM has provided an excelent opportunity for establishing of collaboration with Dr. Anna Paziewska-Harris, which will be extended by publishing the scientific results of the project. The link between both institutions (Slovak Academy of Sciences and KIT BR) also gives the opportunity for potential collaboration on different projects in the future.

Foreseen publications/articles resulting from the STSM (if applicable)

The methods of molecular data analysis and the epidemiological issues discussed during the visit will be the basis for preparation of papers, once the full dataset is avaliable. The papers will be published in peer-reviewed journals, and the rank of the journals will depend on the final outcomes. Possible papers include: „Molecular epidemiology of *Bartonella* spp. in rodents collected in different habitat types in Slovakia" and „The role of flea community composition in establishing *Bartonella* diversity"

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

I hereby confirm that the aims of the STSM entitled "Molecular diagnosis and phylogeny of *Bartonella* spp." (COST-STSM-ECOST-STSM-TD1303-080914-048135) have been successfully accomplished.

Anna Paziewska-Harris

Amsterdam, 26.09.2014 Dr. Anna Paziewska-Harris

Other comments (if any).

I would like to express my gratitute to Dr. Anna Paziewska-Harris as well as to the Host Institution for hospitality and great working conditions during this short mission. I am also grateful to the COST action TD1303 for providing this grant.

26. 09. 2014 Amsterdam, The Netherlands

Jasna Kraljik

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(Applicant and STSM participant)