European Network for Neglected Vectors and Vector-Borne Infections

STSM GRANTEE: Cristian Raileanu

HOST INSTITUTION: ANSES – Laboratory for animal health, Maisons-Alfort, France

HOST: Muriel Vayssier-Taussat

STSM type: Regular (from Romania to France)

COST Action: TD1303, WG 2

DURATION: 2nd of June – 30th of June 2015

Research regarding the prevalence of tick borne pathogens in Eastern Romania

CONTEXT AND OBJECTIVES

Ticks are important vector arthropods of human and animal pathogens, including viruses, bacteria, and parasites, some having zoonotic importance. As information about agents of disease circulating in vectors in Romania is still limited, the aim of the present study was to detect bacteria and viruses with veterinary and zoonotic importance in ticks collected from field vegetation.

Considering that my PhD research refers to tick-borne diseases in Romania entitled "*Epidemiological and etiological research regarding emerging tick-borne infections in eastern Romania*", my goal was to learn new techniques and to gain access to instruments and methods not available in my home institution.

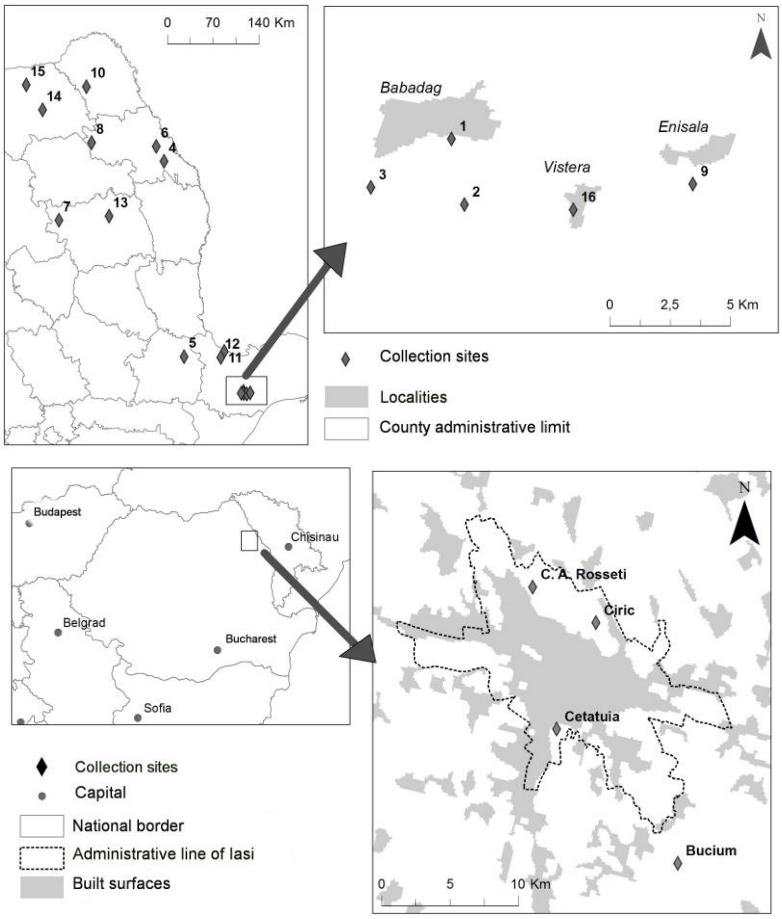
In order to fulfill this I followed two objectives:

A. Identification by molecular detection techniques of pathogens incriminated in producing zoonotic infections
B. Prevalence of known and also novel viruses for Romanian territory in ticks

WORK PLAN

A. <u>Identification by molecular detection techniques of pathogens incriminated in producing zoonotic infections</u>:

The samples to be tested were represented by DNA extracts obtained from questing ticks collected from 20 sites in eastern Romania (figure). The number of samples was 558, each tick being tested individually (the extraction was performed for each tick - at adult and nymph stages).



Associated activities:

- Conduct PCR, QPCR on 558 DNAs extracts.
- Sequence and analyse PCR products

All samples were screened by PCR for presence of DNA from *Bartonella* spp. and *Rickettsia* spp. Detection was carried out using specific primers targeting *gltA* gene for both, *Bartonella spp.* and *Rickettsia spp.* The obtained PCR products (a 380-400 bp fragment for

Bartonella spp. and a 381 bp fragment for *Rickettsia* spp.) were visualized under UV illumination after electrophoresis migration on a 2% gel agarose stained with 0.2 mg/ml ethidium bromide using a 100-bp DNA ladder as a marker. The results showed that 14.3% of tested ticks were positive for infection with *Rickettsia* spp. and 4.1% ticks tested positive for *Bartonella* spp.

Program PCR *Rickettsia* spp

Temp.	Time	Cycles
98ºC	30 s	1
98ºC	10 s	
56ºC	30 s	35
72ºC	30 s	
72ºC	10 min	1
4ºC	∞	

Program PCR *Bartonella* spp

Temp.	Time	Cycles
98ºC	30 s	1
98ºC	10 s	
52ºC	30 s	35
72ºC	30 s	
72ºC	10 min	1
4ºC	∞	

We performed real-time TaqMan PCRs on a LightCycler® 480 (Roche Applied Science, Germany) to test the DNA samples for *Borrelia* spp., *Anaplasma phagocytophilum and Candidatus* N. mikurensis with specific primers and probes targeting *23S rRNA* gene for *Borrelia* spp., *msp2* gene for *Anaplasma phagocytophilum* and *groEL* gene for *Candidatus* N. mikurensis.

Following qPCR program was used to test these pathogens:

Temperature	Time	Cycles
95ºC	5 min	1
95ºC	10 s	
60ºC	15 s	45
72ºC	1 s	
72ºC	∞	

After testing using specific primers and probes for each pathogen there were registered positive ticks for all tested pathogens by qPCR (*Borrelia* spp., *Anaplasma phagocytophilum* and *Candidatus* N. mikurensis)

B. <u>Prevalence of known and also novel viruses for Romanian territory in ticks</u>

Known viruses and also novel viruses were screened in each RNA sample obtained from individual ticks (female, male and nymph stage) collected in eastern Romania.

RNA samples were screened for tick-borne encephalitis virus (TBEv) and EYACH virus by reverse transcription real time PCR assay with specific primers and probes at a concentration of 10 μM. There were samples that showed a positive response for TBEv after rRT-PCR and for confirmation we performed one step RT-PCR with outer primers: FSM-1 and FSM-2 and nested PCR with inner primers: FSM-1i and FSM-2i. All samples showed to be negative for TBEv and EYACH virus.

Foreseen publications/articles resulting from the STSM

The results of this STSM will be published in 1 peer-reviewed journal.

Acknowledgements

I would like to thank COST for funding the Short term scientific mission and for giving me the opportunity for this stage. I especially wish to thank Dr Muriel Vayssier-Taussat, head of VECTOTIQ research team who coordinated with professionalism, ability and enthusiasm my activity durind the stay and also to Sara Moutailler who helped me in gaining important skills and knowledge in molecular biology techniques.

Dr Muriel Vayssier-Taussat
USC BIPAR, INRA, ENVA, ANSES, UPEC
ANSES
14 rue Pierre et Marie
94701 Maisons-Alfort Cedex
Tel 01 49 77 46 55
Email : mvayssier@vet-alfort.fr

Maisons-Alfort, the 3rd of July, 2015

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

I herein confirm the report of Cristian Raileanu regarding the COST-STSM-ECOST-STSM-TD1303 in France.

Sincerely,