European Network for Neglected Vectors and Vector-Borne Infections

BERNHARD-NOCHT-INSTITUT FÜR TROPENMEDIZIN

SHORT TERM SCIENTIFIC MISSIONS (STSM) SCIENTIFIC REPORT

Beneficiary: Edina Török, PhD student
COST STSM Reference Number: COST-STSM-TD1303-29043
Research Project Title: Investigate on the mosquito (Diptera, Culicidae) biodiversity and their pathogens in the metropolis Cluj-Napoca, Romania
Home Institution: Romanian Academy Institute of Biology – Bucharest (Romania)
Host Institution: Bernhard Nocht Institute for Tropical Medicine, Arbovirology Group (Germany)
Host: Univ.-Prof. Dr.med. Dr.med.habil. Jonas Schmidt-Chanasit
Period: 04/10/2015 to 28/11/2015

Short Introduction

Many human and animal diseases can be caused by viruses transmitted by arthropods (arboviruses), thus studying them have a growing importance in many European countries, including Romania. Globalization and climate changes create favorable conditions for several exotic pathogens to expand their original distribution ranges toward to the temperate regions. A series of recent works forecasts the re-emerging of once eradicated diseases in Europe or the introduction of some new ones, due to an important supply of pathogens and their vectors, like malaria mosquitoes, from the surrounding regions (Africa or Asia). The aim of this study was to identify mosquito species and potential mosquito-borne viruses (moboviruses) focusing on different wet ecosystems in Cluj-Napoca, the second biggest city in Romania (population 324 576 Cluj County Regional Statistics Directorate. 5 July 2013). Using standard collection methods we performed a longitudinal survey in order to identify the mosquito communities in four ecosystems surrounding the city, and to detect the circulating moboviruses using molecular methods.

The present survey should serve as an early warning system for public health, and is of great interest in epidemiology, by detecting possible new pathogens and by understanding their ecology and estimates the impact on public health.

Aims of the study

The major aim of our study was to identify mosquito species from different natural and artificial ecosystems in Cluj-Napoca and its surroundings, and their role as potential vectors of some important viruses.

During our investigation we focused on two main directions:

1. Identification of potential habitats of the Culicidae communities from Cluj Napoca and its surroundings, and characterization on its species level composition

2. Detection of potential moboviruses in Culicidae communities and evaluate their pathogen risks

Materials and Methods

In our present study we applied a longitudinal survey to detect Culicidae communities from different ecosystems in Cluj-Napoca and in the neighbor NATURA 2000 sites ("Făgetul Clujului – Valea Morii" forest habitats and "Eastern Hills of Cluj" hayfields).

Mosquitoes were sampled from June to September, from 7 study sites from different wet or humid ecosystems, as follows (Fig 1.):

1. The Somes River side near "Eastern Hills of Cluj" hayfields
2. Riparian vegetation along a secondary channel of Somes River near "Eastern Hills of Cluj "hayfields
3. Urban lake
4. Fishing lake
5. Water Treatment Station in center of Cluj Napoca
6. Botanical Garden
7. Forest habitat in "Făgetul Clujului – Valea Morii"

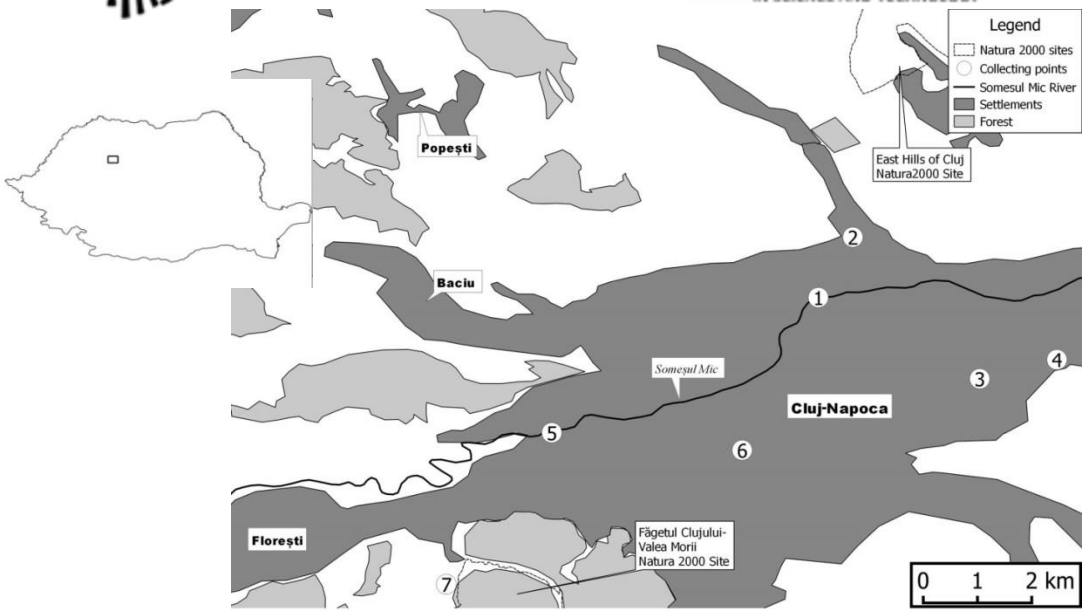

Fig.1 Collecting sites in Cluj-Napoca

We used handmade Gravid Traps (GT), following the standard protocols. The GT traps are designed to catch mostly gravid female mosquitoes in search for ovipositioning sites. The traps were equipped with a dark colored open-topped container filled with a water-based infusion. Above the water surface a fan was fixed to produce an upward air circulation. 4 D/ Mono batteries with 1.5 V were used as power supply. Gravid females attracted by the infusion's decaying material and open water surface were sucked in the net and collected regularly (Fig 2.). The GT were used 17 weeks, the mosquitoes were collected weekly and preserved frozen at -20 Cº prior for identification.

Fig.2. Collecting habitats and Gravid traps in Cluj-Napoca

The Culicidae species were identified in laboratory conditions based on morphological characteristics. The potential moboviruses from the collected Culicidae species were screened following the classical molecular methods (PCR):

- Homogenization and extraction of genetic material (DNA and RNA) from mosquitoes using special MagMAXTM Pathogen RNA/DNA Kit protocol using KingFisher Flex extraction machine
- Application of well-established classical pan-PCR methods for detection of members of families or genera of moboviruses (*Flaviviridae*, *Orthobunyaviridae*, Alphavirus, *Rhabdoviridae* and Phebovirus) (pan-PCR: pan-flavivirus, pan-Orthobunyavirus, pan-Phlebovirus, pan-Alphavirus, pan-Rhabdovirus)
- experimental infection of the mosquito homogenates on cell culture (*Aedes albopictus* clone C6/36 cells)

The identification of the potential viruses was performed in the laboratories of the Arbovirology Group, Bernhard Nocht Institute for Tropical Medicine (BNITM) in Hamburg.

Results and Discussions

During the present study we collected 728 specimens belonging to 14 different mosquito species (Table.1.). The majority of the mosquito materials were collected in 6 different collection sites. The largest number was collected in the Botanical Garden (165 specimens) and the Forest habitat (155 specimens). In the perimeter of the Water Treatment Station site we were not able to identify any Culicidae species, probably because of the periodical mosquitoes control management of the place.

The total number of 728 Culicidae individuals collected are important in respect to the short period of our investigation, limited mostly to summer time (from June to September), resulting in a number of 14 Culicidae species, which represents 73.68% of species identified from Transylvania. This is a relatively high number reported to the recent work of Nicolescu et al., who identified between 2002 and 2003 a number of 19 Culicidae species. Four species, *Culiseta alaskaensis*, *Ochlerotatus cataphylla*, *Ochlerotatus punctor*, *Coquiuettidia richiardii* are reported for the first time for the fauna of Transylvania, based on our results. This high number of Culicidae species identified in the area of Cluj Napoca is in

relation with the variety of some suitable habitats (mostly wet habitats with important organic supply).

Table1. Culicidae species identified in the present study, with numbers of individuals, sites and months

	Culicidae species	**Number of specimens female/male**	**Collecting site**	**Collecting months 2015**
1	*Aedes (Aedes) cinereus* Meigen 1818	10f	4	September
2	*Aedes (Aedes) vexans* (Meigen, 1830)	13f	4	June
3	*Anopheles claviger* (Meigen, 1804)	16f	3	September
4	*Anopheles (Anopheles) maculipennis* complex Meigen, 1818	157f,2m	2, 7	July, August
5	*Culex (Culex) pipiens* Linnaeus, 1758	164f/154m	1, 2, 3, 4, 6, 7	June, July, August, September
6	*Culex (Culex) territans* Walker, 1856	75f	1, 3, 4, 6	June, July
7	*Culiseta (Culiseta) alaskaensis* (Ludlow, 1906)	11f	4	September
8	*Culiseta (Culiseta) annulata* (Schrank, 1776)	11f,3m	4	July, August, September
9	*Ochlerotatus (Ochlerotatus) annulipes* (Meigen 1830)	3f,2m	3	August
10	*Ochlerotatus (Ochlerotatus) cantans* (Meigen, 1818)	3f, 3m	7, 4	June
11	*Ochlerotatus (Ochlerotatus) caspius* (Pallas, 1771)	9f	7	September
12	*Ochlerotatus (Ochlerotatus) cataphylla* (Dyar, 1916)	5f	3, 4	September
13	*Ochlerotatus (Ochlerotatus) punctor* (Kirby 1837)	32f, 5m	7	June
14	*Coquiuettidia (Coquillettidia) richiardii* (Ficalbi, 1889)	48f,2m	1, 3, 4	July, August, September

Among the different Culicidae species identified in this study, 10 species (*Aedes vexans*, *Anopheles claviger*, *An. maculipennis* complex, *Culex pipiens*, *Cx. territans*, *Culiseta annulata*, *Ochlerotatus annulipes*, *Oc. cantans*, *Oc. caspius*, *Coquiuettidia richiardii*) are known to be potential vectors for a series of mosquito borne viruses [(e,g, Sindbis Virus (*Togaviridae*), West Nile Virus, Usutu Virus (*Flaviviridae*), Tahyna Virus (*Banyaviridae*)], already reported from different areas of Europa.

During the present study, we were not able to detect any of the viruses mentioned above, having no positive results applying pan-PCR.

Despite the fact that we were not able to identify viruses in the collected mosquito material using pan-PCR methods, we inoculated the homogenized samples on C6/36 *Ae. albopictus* cells cultures. After two weeks of incubation we analyzed we founded evidences of cytopathogenic effects (CPE) in 16 samples from which we extracted and analyzed genetic material again with pan-PCR. The results showed the presence of non-specific mosquito viruses, only.

In conclusion, we found 14 mosquito species (728 specimens) in Cluj-Napoca, from which four species are recorded for the first time in Transylvania and represents 73.68% of the Culicidae fauna recorded from here.

During our investigation we were not able to identify any moboviruses in the investigated Culicidae material, despite the important human population concentration in Cluj Napoca and the presence of some suitable habitats hosting a large and diverse mosquito community. There are an important number of suitable wet habitats in Cluj Napoca and its surroundings (lake ecosystems, the presence of the Somes River), therefore the lack or the very low level of virus infection of the studied Culicidae community could be associated with an only sporadic presence of migratory birds.

Focusing on my scientific carrier, this fellowship was very useful because I was initiated in mobovirus identification technics and learned from the highly qualified colleagues from the Bernhard Nocht Institute for Tropical Medicine (BNITM). They have a long tradition in mosquito borne viruses identification and data analyses. I acquired several new laboratory methods which I will be able to apply in future when I will be back in the laboratories of my home Institute. The results generated during this study will be published in a specialized impact factor journal.

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

I herein confirm the present report regarding the Edina Török COST-STSM-TD1303-29043 in Hamburg.

Head of the Arbovirology Group
Prof. Dr. med. Dr. med habil Jonas Schmidt-Chanasit, MD

Applicant and STSM participant:
Edina Török
PhD student

Report done on December, 10th, 2015