



EVD-LabNet Newsletter 5, March 2023

About EVD-LabNet

EVD-LabNet is a multi-disciplinary network of expert laboratories. Its aim is to strengthen Europe's laboratory capacity and capability to respond to emerging, re-emerging and vector-borne *viral* disease threats. The network laboratories are located in the EU/EEA, EU pre-accession countries, and is being expanding to the EU neighborhood policy countries. These laboratories have a strong basic and/or translation research competence in virology and human (reference) diagnostics and/or experience in diagnostic test development for viral pathogens. The network is a continuation of ENIVD that was founded about 30 years ago and has been known as EVD-LabNet since 2016.

Tick-borne encephalitis virus phylogeography study.

To fill a knowledge gap on phylogenetic and dispersal patterns of tick-borne encephalitis virus (TBEV), a large-scale collection and analysis of TBEV samples/genomes from different countries in Europe and from distinct foci within countries was conducted. For this study 253 TBEV genomes from across Europe were analysed, the majority of sequences being generated as part of this study. On a European scale there is limited geographical clustering, indicating several long-range dispersion events of the virus. In a small scale however, the typical very focal occurrence of distinct TBEV subclades is evident. A major radiation event in the ancestral lineages of both TBEV-Eur clades occurred approximately 700 years ago, and led to the emergence of distinct TBEV-Eur sub-lineages of clade B. TBEV-Eur clades in remote foci have evolved in isolation typically for decades compatible with long-range dispersion events, followed by establishment and continuous circulation in favourable environments. The pattern is consistent with the hypothesis that migratory birds act as distributors of TBEV to new areas.

While the observed strain diversity seems to fall within the scope of detection by current diagnostic methods and vaccine efficacy according to current knowledge, the spread of TBEV-Sib strains in *Ixodes ricinus* warrants monitoring. However, the current TBEV taxonomy is incompatible with the phylogeny, as TBEV and Louping ill virus (LIV) are classified into two separate species although TBEV-Eur and LIV have a more recent common ancestor than the current TBEV species. The species *Tick-borne encephalitis virus* is paraphyletic suggesting the current taxonomy should be revised. The study involved multiple EVD-LabNet partners and a manuscript is being prepared for submission to a peer-review journal.

EU case definition review

EVD-LabNet supported the ECDC through the review of a number of EU case definitions for human virus infections including human disease caused by the following viruses, virus genera or virus families: *Arenaviridae*, Crimean-Congo hemorrhagic fever virus, chikungunya virus, dengue

virus, *Filoviridae*, *Orthohantaviruses*, *Lyssaviruses*, mpox virus, Rift Valley fever virus, TBEV, West Nile virus, variola virus, yellow fever virus and Zika virus. Drafts of the revised case definitions were sent around to all EVD-LabNet members asking for comments. Thank you to all those who participated and provided support. The list of diseases under EU surveillance and the revised EU case definitions are currently with the European Commission for further comments.

Access EVD-LabNet activities for ENP-countries.

To involve laboratories from ECDC's European Neighborhood Policy (ENP) countries in EVD-LabNet activities, an amendment of the EVD-LabNet framework contract was signed to finance participation of such laboratories in upcoming EVD-LabNet EQA's. ENP countries are: Algeria, Armenia, Azerbaijan, Belarus¹, Egypt, Georgia, Israel, Jordan, Lebanon, Libya, Moldova, Morocco, Palestine, Syria, Tunisia, and Ukraine. As a first step of integration, laboratory representatives from these countries will be invited to attend virtually the EVD-LabNet annual network meeting.

2023 upcoming activities

For 2023 the following events are planned.

External quality assessment. In 2023, EVD-LabNet is planning an EQA covering molecular detection of globally significant mosquito viruses, in different clinical specimens, since these viruses are frequently diagnosed among European travelers and in limited local transmission chains in southern Europe. This EQA will also be open for specific ENP-country laboratories.

Webinars – We anticipate offering two webinars in 2023 (spring and fall) based on topics identified within the network. The first webinar will be held on 20 March 2023 and focuses on hot topic updates:

- Dennis Tappe, Bernhard Nocht Institute for Tropical Medicine (BNITM) : Borna disease virus 1 (BoDV-1) situation in Germany
- Vítor Borges, National Institute of Health Dr. Ricardo Jorge (INSA): Multi-country outbreak of mpox virus: genetic clustering and transmission dynamics
- Maria (Sheny) Morales-Betoulle, USA Centers for Disease Control and Prevention (CDC): Sudan ebolavirus epidemic review
- Sandra Junglen, Charité Univ. Berlin (CUB): Ngari virus circulation in Kenya

Trainings – We anticipate having three training events in 2023. Two will be virtual and one face-to-face event. These do not yet have a date attached to them, but the anticipated quarter of the year they are projected to occur will be listed next to them below.

- o Training 1 (Q2) : Basic Laboratory Methods for Epidemiologists - Virtual
- o Training 2 (Q3) : Sequencing, Molecular Epidemiology and Bioinformatics – Virtual

¹ Financial cooperation with Belarus has been suspended as of March 2022.

- o Training 3 (Q4-2023 or Q1-2024) : Virus Culture, Characterization, and Neutralization – Face-to-Face

Annual Network Meeting. We anticipate offering the EVD-LabNet annual meeting for 2023 as a hybrid meeting (both in-person and online). The event will be in Padua, Italy 30-31 May 2023. On site attendance will be supported by EVD-LabNet for one participant per member institute. Please look for further information coming in the next weeks.

Highlights of network activities in period 2021-2022.

EVD-LabNet activities in 2021-2022 were highly impacted by the COVID-19 pandemic with the management team and members fully involved in the (inter)national response to the pandemic while restrictions in society were in place. The biggest losses for the network were the absence of the annual face-to-face scientific network meetings and training sessions. Despite the SARS-CoV2 - related restrictions, EVD-LabNet coordination managed to organize various capacity-building activities including virtual meetings, one training and external quality assessments. The network members are highly valued for their faithful participation in the network activities despite the circumstances making it extremely difficult to find capacity to do so.

External Quality Assessments.

Two external quality assessments (EQA) were conducted by the network in collaboration with the European Virus Archive (EVAg).

1. Molecular and serology-based detection of rodent-borne viruses with a focus on European orthohantaviruses and lymphocytic choriomeningitis virus (LCMV). Overall, 25 laboratories from 20 EU/EEA and EU-enlargement countries participated of which 16 in the molecular detection assessments and 20 in the serology assessment. Individual results in the context of the anonymised total of results were reported back to the participants in a timely manner to enable corrective actions if needed. The results showed improvements in the molecular detection of orthohantaviruses are needed, while LCMV detection was above 90% for the participating laboratories. Serological diagnosis of orthohantaviruses relied mainly on commercial assays and the performance was good (>90%). A full report will be published for a wider audience in the near future. The organisation of the EQA was a collaboration between Aix University Marseille, University of Helsinki, University of Marseille, RIVM and RKI.
2. Molecular detection of emerging alphaviruses. The EQA recruited 23 participants. Individual results were reported back to the participants to support timely corrective actions if needed. In-depth analysis is currently ongoing and will be presented in a full report for a wider audience. The EQA was a collaboration between Aix Marseille University, ANSES-Maison-Alfort and RIVM.

Training.

Scientific webinars. Three online scientific webinars (2 hours each) were organized with presentations of renowned experts in the field and open to network members and the wider scientific community. The three webinars focused on emerging alphaviruses, emerging rodent-borne viruses and tick-borne encephalitis respectively. The webinars covered, in One Health context, the epidemiology, clinical manifestations, diagnostics and immune responses to these emerging viruses.

In-depth training course. An online in-depth course was organized on emerging alphaviruses in February 2022.

Scientific support.

Factsheets. Four factsheets were written by various members of the network i.e. Aix Marseille University, Aristotle University of Thessaloniki, Erasmus MC, Public Health Institute of Croatia and RIVM. The topics were [Crimean Congo hemorrhagic fever](#), [Toscana virus infection](#), [Marburg virus disease](#) and [nipah virus disease](#).

Short survey. A short survey on the impact of COVID-19 pandemic on arbovirus diagnostics was conducted among member laboratories. With SARS-CoV-2 putting pressure on laboratory structures worldwide, we aimed within EVD-LabNet to evaluate the pandemic impact on the continuity of diagnostics of two of the most common arboviruses in Europe: TBEV and WNV. Thirty-two laboratories in 25 countries responded. The results of the survey show a complex impact of the pandemic on TBEV/WNV diagnostics in Europe. An observed decrease in the number of received samples might also be attributed to lockdowns and do not necessarily represent reduced capacity in the health care chain. Highlighted issues were decreased participation in EQAs, training, research, surveillance and delays in other quality control activities like updating protocols.

Systematic review. A systematic review of literature on the value of urine as sample for flavivirus RNA detection has been initiated. The emergence of different pathogens during the last decades raised some questions on reliability of testing of different body fluids for identification of cases. To date, no systematic reviews have been conducted addressing the usefulness of urine for diagnosis of flavivirus infections. Therefore, a literature search was performed on three databases (PubMed, Embase and Scopus) up to May 2022, focusing on the sensitivity of urine *versus* other clinical samples in relation to the techniques used. Data collected is currently being analyzed for peer-reviewed publication. The data will help understand the diagnostic value of this non-invasive specimen during the course of infection, thus guiding choices for laboratory diagnosis and laboratory case definitions.

Scientific project. See tick-borne encephalitis virus phylogeography study mentioned above.

Upcoming activities.

- 20 March 2023 webinar on current hot topics. No registration needed. [Webinar link here](#).
- 6th Annual EVD-LabNet meeting. University of Padua, Italy. 29-31 May 2023.
- Training – Laboratory methods and techniques for Epidemiologists – Q2 (April-June)

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