Report on completed EPIZONE short-term mission 2026

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PhD project title: Vector-borne viruses, focus on diagnostics for orthoflaviviruses.
Supervisors: Principal supervisor Lars Erik Larsen, primary co-supervisor Graham J. Belsham, co-supervisors Thomas Bruun Rasmussen and Ann Sofie Olesen.
Workplaces in Denmark: Department of Veterinary and Animal Sciences, University of Copenhagen and Department of Virology and Microbiological Preparedness, Statens Serum Institut.
Period for stay: 07.04.26 – 26.06.26
STM host institution: ANSES, Laboratoire de Santé Animale, UMR Virologie ANSES-INRAE-EnvA, France
STM hosts: Gaëlle Gonzalez and Camille Migné

Purpose of stay

Thanks to the EPIZONE Short-Term Mission grant, I was able to perform a three-month research stay as part of my PhD study from April to June 2026. The host institution was *L'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail* (ANSES) in Maisons-Alfort, France. More specifically, I was in the "Neurovirology and equine zoonoses (ZEN)" group that is a part of the joint research unit "UMR Virologie. This group focuses on zoonotic and epizootic viruses of major importance for human and veterinary public health. The ZEN group functions both as a national reference laboratory and as a European Union reference laboratory for several important veterinary viruses, including West Nile virus (WNV).
The purpose of the stay was to strengthen my PhD project on veterinary orthoflavivirus diagnostics by acquiring advanced methodological expertise and generating novel data in collaboration with orthoflavivirus experts at ANSES. In particular, the objectives were to receive training in virus isolation techniques and virus neutralization tests (VNT) and to gain insights into multiplex serological methods such as Luminex. In addition, the work aimed to contribute to increased knowledge of these viruses and to improve diagnostic preparedness in Denmark. Lastly, the purpose was to strengthen existing collaborations between the institutions and to provide the basis for future joint projects and scientific publications.

Work achievements and experiences

At the beginning of my stay, I was introduced to the laboratories, facilities and to the members of the unit. Each researcher presented themselves and their work, and I got a broad overview of the activities and capabilities of the virology department at ANSES.

During the first two weeks, I became familiar with the group and the laboratories, and I primarily followed the laboratory technicians to gain an understanding of the analyses

conducted as part of the role of the ZEN group as national and European Union Reference Laboratory for WNV and other equine encephalitis viruses. We compared protocols and discussed sample analysis techniques for assays I was already familiar with, such as the IgG ID Screen® Flavivirus Competition ELISA, ID Screen® West Nile IgM Capture, and RT-qPCRs. I also saw how VNT is performed and how it is used in the diagnostic workflows in the ZEN group. In the second week, I presented my PhD project at a joint research group meeting. The presentation went well, and I received interesting questions from the audience.

After the first two weeks, we began processing and analysing samples that I had brought from Denmark to ANSES. For the research stay, I brought two sets of samples; tissue and swab samples from Usutu-positive blackbirds collected in 2025 (including heart, lung, liver, spleen, kidney, intestine, feathers, cloacal, and oropharyngeal swabs) and serum samples from zoo birds. The experimental plan for the blackbird samples was initially to test all tissues and swabs using RT-qPCR to detect USUV RNA, and subsequently to perform virus isolation from selected organ samples and all swab samples. For the zoo bird sera, the plan was to analyse the samples using serological methods, namely VNT and Luminex.
We started working with the blackbird samples, initially performing qPCR on the organs and swab samples, which was highly successful, and subsequently virus isolation which also yielded satisfactory results. Results from the organs and swabs have already contributed to an abstract submitted for the EPIZONE annual meeting in Bern in September 2026. The feather samples were not analysed during the stay at ANSES, but it is planned to analyse them in Denmark during 2026.

After analysing the samples obtained from the blackbirds, we started working on the zoo bird samples. In Denmark, they had already been tested positive for antibodies against orthoflaviviruses using a conventional ELISA, so the next step was to test for antibodies against specific viruses. For this, we used the VNT assays specific for WNV or Usutu virus (USUV), which showed that some samples contained antibodies specific for WNV while other samples contained antibodies specific for USUV. Finally, some samples were inconclusive in the VNTs (due to cross-reactivity or possible co-infections). For the final week, we had planned to analyse the zoo bird samples using the Luminex assay. However, due to a serious heatwave in France, it was unfortunately not possible to perform this analysis, as the assay is temperature-sensitive and the laboratory did not have sufficient air conditioning to cope with the high temperatures. The results from the zoo birds will contribute to a collaborative project involving two different zoos in Denmark and it is planned to result in a publication.

In addition to the planned experiments, I also gained hands-on experience with Vero NK cells, which are the preferred cell line for USUV and WNV work at ANSES. I had only very limited experience working with cells in general, but I now feel confident working with

this cell line, and I expect that I will try to work with them in Denmark as well. I also gained further experience in virus titration methods. I had previously performed a single $TCID_{50}$, but during the stay I conducted several titrations for both USUV and WNV, and I was also trained in plaque-forming unit assays.

Another very interesting experience was attending the 18th workshop of the European Union Reference Laboratory (EURL) for equine diseases hosted by ANSES, which focused on arthropod-borne equine encephalitis viruses including WNV (and USUV). Differences in the geographic spread of these viruses, emerging trends in Europe, the latest laboratory diagnostic methods, and much more were discussed and I really saw the value of working together and sharing knowledge and experiences.

Lastly, I attended the weekly group meetings, during which diagnostic results and recent clinical cases were presented and strategies for handling positive cases were discussed. This provided me with interesting insights into how a European Reference Laboratory manages WNV-positive cases.

Personal experiences

Visiting ANSES and working in the ZEN group has truly been a great experience. The group welcomed me with warmth and dedication, and I quickly felt like an integrated member of the group. I do not speak French, but it all worked out just fine as my colleagues in the group did their best to translate protocols into English, conduct group meetings in English and speak English during lunchbreaks etc. I have long dreamed of trying to live and work/study in another country, so this stay has also been a very meaningful and memorable experience for me in this way. I manged to learn just a little French and gained a sense of French culture and that has also been really fun.

Conclusion

Overall the research stay has been successful, and the initial goals of the stay were for the most part achieved. There were a few obstacles, such as a heatwave that prevented us from using the Luminex machine and insufficient time to analyse the feather samples, but I still managed to acquire technical skills, learn new techniques, generate novel data, establish strong professional connections, and mature as an early-career scientist. The stay was as hoped, and it has made an impact on me both professionally and personally.

In the beautiful old lecture hall shortly before I presented at the joint research group meeting.

Suited up and ready to work in the biosafety level 3 laboratory.

Photo of the ZEN group