Report on completed EPIZONE short-term mission

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PhD project title: Animal coronaviruses - detection and characterization
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Workplace: DTU Vet, Lindholm, Denmark and DTU Vet, Kongens Lyngby, Denmark
Period for stay abroad: 01-09-2019 – 30-11-2019
Host institution: IZSLER, Brescia, Italy
Host institution hosts: Dr. Beatrice Boniotti and Dr. Antonio Lavazza

Purpose of stay

Thanks to the EPIZONE short term mission grant, I was able to go on a 3 month research stay as part of my PhD studies from September to November 2019. The host institution was Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER) in Brescia, Italy. Initially the plan was to work both in the Virology department led by Dr. Antonio Lavazza, who was my contact for the stay and in the Genomic department with Dr. Beatrice Boniotti. The purpose was to validate serological assays using recombinant PEDV structural proteins (established at DTU Vet) using serum samples available at IZSLER and to use PEDV positive RNA samples for validation of three PEDV and SeCoV duplex RT-qPCR assays.
However, shortly before the beginning of my stay, we decided for biosafety reasons (I am pregnant) that I would not be allowed to work in the laboratories in the Virology department. Therefore, Dr. Beatrice Boniotti offered to host me for the whole stay in-stead. For this reason, the sole focus of my stay became development and validation of PEDV and SeCoV duplex RT-qPCR assays. Validation of serological assays had to be postponed until I was back at my workplace in Denmark bringing Italian serum samples from swine with me.

Work achievements and experiences

In the beginning of my stay at IZSLER, I was introduced to the people working in both the Virology and Genomic departments. I had several tours of different parts of the Institute including the Virology department, where I heard about species sorting of mosquitos and the ongoing screening for West Nile Virus and other viruses. I was also given a short introduction to the electron microscopy facilities, which are used as an addition to e.g. NGS for characterization of new pathological agents. I had a tour of the large cell culture facility, with only dedicated staff working here, providing established cell lines and primary cells for customers within and outside of IZSLER. And finally, I saw the biobank of veterinary resources (BVR) with large liquid nitrogen cryo containers and heat controlled rooms for e.g. virus and bacterial isolates kept for future preservation and for use in research.

After a few weeks at IZSLER, the two departments of Virology and Genomics were invited to hear a presentation about my PhD study with previous activities and results and planned activities at IZSLER. It was well received and prompted interesting questions and suggestions from the audience e.g. regarding some of the previous animal experiments and choice of protein expression systems in mammalian or insect cell culture. Afterwards, some of the colleagues at IZSLER asked me for introductions to the programs I use for data analysis of NGS data, and now they are using the same.

The first activities in the laboratories in the Genomic department were to generate in-vitro transcribed RNA of partial N and S gene of a recent Italian strain of Swine Enteric Coronavirus (SeCoV) for the subsequent use in the PCR validation. I did this together with Alice Papetti, who is also developing an RT-qPCR assay for porcine coronaviruses. We used TOPO cloning, which I was already familiar with, but other techniques, instruments and workflows were new to me e.g. the NanoQuant instrument, the use of a millipore filter for filtering of salts after the TOPO reaction and before electroporation of cells, the use of exonuclease treatment of PCR products instead of column purification before sequencing, the use of a resin instead of spin column purification of the sequencing reaction, in-vitro transcription of RNA and the calculation of copy numbers for concentration determination.

For the main work of RT-qPCR validation, I had to learn how to use another PCR machine and analysis program from what I was used to (Bio-Rad CFX in-stead of Stratagene Mx3005-P). The PCR runs for validation of the duplex RT-qPCR assays were set up with ongoing feedback from Beatrice Boniotti and Alice Papetti, so that all the necessary validation parameters were determined, and so that the test results could be compared to the porcine coronavirus multiplex assay in development at IZSLER. It is planned that the validation of both their multiplex assay and my duplex assays will be published in a joint paper.

Personal experiences

From the first day at IZSLER, I felt very comfortable in the new work environment. Everybody has been very friendly and helpful and although the English language can be a challenge for some, my colleagues have tried to include me in conversations and translate a lot of Italian for me. My colleagues also arranged a social event in my honor with a Sunday dinner including families at a restaurant in Brescia, and gave me a beautiful gift basket at the end of my stay. I think this warm and welcoming attitude has been very important for my overall experience, and it makes me reflect on the way we include (or don't include) non-Danish speakers at workplaces in Denmark.

Another important thing for my personal experience was that my husband and young son were able to come along to Brescia for 3 months. The EPIZONE short term mission grant was crucial for the coverage of accommodation during this time.

Conclusion

IZSLER has been a good collaboration partner for DTU Vet for many years and my supervisors have worked previously on projects together with Dr. Antonio Lavazza, Dr. Beatrice Boniotti and others from IZSLER. Now that I have been there and have gotten to know a lot of people, I think the relationship has been strengthened and that there are more possibilities for collaboration in the future. In the near future this will be in the form of a joint publication. The external research stay was well timed with other events, as my workplace in Denmark has recently changed and laboratory facilities and materials are not ready for use yet, but by going abroad I could still work in the laboratory, have access to PEDV and SeCoV field samples and get closer to finishing my PhD studies.


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