

Report of COST-STSM -FA1408-040416-072835

Training of new technique MALDI-TOF MS used in investigations on protein profile of *Trichinella* isolates.

The short term scientific mission “Training of new technique MALDI-TOF MS used in investigations on protein profile of *Trichinella* isolates” took place in National Reference Laboratory (NRL) for *Trichinella* of the Federal Institute for Risk Assessment (BfR) between 03.04 - 10.04.2016. The training was conducted by Peter Bahn under the supervision of Dr. Anne Mayer-Sholl and Dr. Karsten Noeckler.

For fast identification of *Trichinella* species and investigation of infection chains, mass spectrometry matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometer (MALDI-TOF) has recently been developed at the BfR. MALDI TOF MS is an ionization technique allowing mass spectrometry analysis of biomolecules such as proteins. The method is based on the recognition of specific proteins of the microorganisms. As both the protein composition of each organism and the MALDI-TOF MS spectrum are unique, the obtained spectra can be compared with a database of known microorganisms and the sample identified with high confidence.

The purpose of the training was to learn the technical aspects of MALDI-TOF, including protein extraction techniques. Furthermore, an additional aim of the STSM was the analysis of spectra measured from *Trichinella* field isolates from the West Pomeranian region in Poland in comparison to spectra of isolates from Mecklenburg–Western Pomerania, Germany.

Trichinella larvae were isolated at the National Veterinary Research Institute laboratory in Poland, from 29 wild boars and 2 red foxes and *Trichinella* species identification was performed by multiplex-PCR. Prepared isolates were transported to the BfR in 96% ethyl alcohol.

Proteins from the collected field isolates of wild boars and red foxes from Poland were extracted at the NRL for *Trichinella* (BfR). The protein was extracted from ten pooled larvae per isolate with 10 mg zirconia/silica beads (0.5µm) and 100µl 70% formic acid in a 2 ml Eppendorf tube. The samples were sonicated on ice for 3 min, and then 100µl acetonitrile (100%) were added and sonicated again under the same conditions. The suspension was centrifuged at 14.000g for 3 min at room temperature, and subsequently 150µl of supernatant was collected for protein concentration in a vacuum centrifuge. One µl of each sample was spotted onto the target plate nine times (Bruker Daltonik, Bremen, Germany), air dried and then overlaid with 1µl of saturated α-cyano-4-hydroxy-cinnamic acid matrix solution. After complete drying, the target with spotted samples and the Bacterial Test Standard for calibration were inserted into the MALDI TOF MS equipment. A total of 27 spectra per specimen were obtained from nine spots where each spot was measured three times. The quality of each spectrum was assessed with FlexA-nalysis 3.4 software.

The spectra from Polish field isolates were compared with the master spectra library (MSP) generated at the BfR using Flex Analysis software. Findings confirm that species identification of larvae was possible by means of MALDI TOF MS and in accordance with results previously gained by multiplex-PCR. All obtained spectra from examined isolates were assessed on species level with high likelihood. The investigations proved the usefulness of MALDI TOF MS for species identification of *Trichinella* field strains. Detailed comparison of the spectra of the field *Trichinella* strains from West Pomeranian region of Poland with spectra of *Trichinella* isolates from Mecklenburg–Western Pomerania, Germany showed some differences in protein profiles on species level. This finding could indicate the existence of different strains of *Trichinella spiralis* or *Trichinella britovi* in Poland and Germany. However, further detailed investigations should be conducted for proving this hypothesis.

In conclusion, the training was successful in terms of the established goals. The STSM visit has successfully contributed to know-how transfer on MALDI TOF MS and improving collaboration between NVRI and BfR on typing of foodborne parasites such as *Trichinella*. The MALDI TOF MS method will in the future be implemented for the investigations of *Trichinella* isolates in the Polish NVRI laboratory. Additionally, the collaboration of scientists from NVRI and BfR in the basic approach of expanding of master spectra library and examination of field strains from Poland and Germany using MALDI TOF MS will be continued in the nearby future.