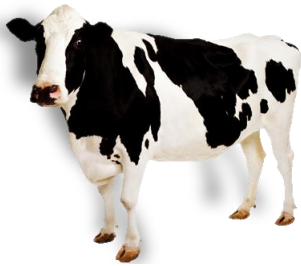


VET-TWIN Newsletter 01/2017



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Trainings at DTU and BfR and Short-term missions

MSc. Anna Gierak from the Department of Epidemiology participated in the training on quantitative microbiological risk assessment, from 11th to 22nd July 2016 at the Federal Institute for Risk Assessment in Berlin. The topics covered theoretical aspects of hazard identification, hazard characterization and risk management as well as practical exercises of model adjustment to real data. Moreover, the useful software which allows making ad hoc analysis of spread of diseases was presented. This software will be used to assess the risk of ASF spread. MSc. Anna Gierak participated also in a short term mission on modelling infectious

diseases in DTU in Copenhagen (21st November to 2nd December 2016). Main purpose of this mission was the training on the mathematical models of ASF and FMD spread on the basis of differential equations using the R environment. Moreover, she had the possibility to discuss with DTU experts the availability of necessary data.

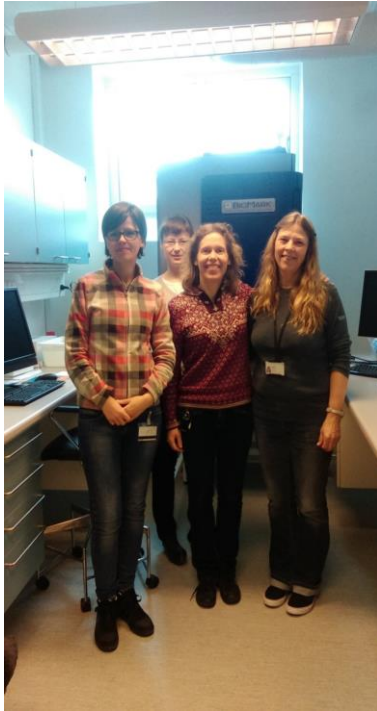
On 5th - 9th September 2016 three delegates from National Veterinary Research Institute in Pulawy: dr Anna Orłowska, dr Samanta Jowita Niczyporuk and dr Ewelina Czyżewska-Dors attended the qPCR workshop focused on application of real time polymerase chain reaction (real-time PCR) for the detection of animal diseases. The training took place at the Danish Technical University - the National Veterinary Laboratory in

Copenhagen, and was organized by dr Charlotte Kristiane Hjulsgaard and dr Jesper Schak Krog. The training included some theoretical lectures upon the introduction and optimization of real-time PCR and multiplex qPCR (primer and probes design, application of LNA nucleic acids, T_m calculation and quantification using standard curves). The attention was paid not only to the benefits of the application of real-time PCR assay, but also to the disadvantages and pitfalls of the technique. The practical workshop involved the introduction of Lab work system organization in DTU - from getting the samples in the lab to issuing the results, initial samples preparation and introduction of automated machines for nucleic acid extraction (QIA Symphony, QIAcube) and qPCR set-up (QIAgility). The trainees participated in quantification of PCV2 in swine serum samples and optimization of multiplex qPCR dedicated to four bovine pathogens: Bovine respiratory syndrome virus (BRSV), Bovine parainfluenza-3 virus (BPI3), Bovine coronavirus (BCoV) and *Mycoplasma bovis* (*M. bovis*).

The next training on next generation sequencing and bioinformatics was held on 8th - 19th August 2016 in DTU Vet - National Veterinary Institute with two participants from NVRI - MSc Edyta Świętoń and Arkadiusz Bomba, and two participants from DTU - dr Mikael Lenz Strube and Sophia Rasmussen. The training was divided into two parts: laboratory work covering sample preparation and bioinformatics/statistical analysis. The laboratory workflow focused on preparation of fecal samples from wild birds for 16S rRNA sequencing. The

bioinformatics part of training included introduction to Linux environment and programming languages, analysis of existing data sets on 16S rRNA sequencing using BION package and statistical analysis with the use of R studio. The complexity of training allowed the trainees to familiarize themselves with a wide range of techniques used in NGS - from preparation of samples to primary analysis of raw data and secondary analysis of statistical relationship.

An interesting short-term mission was attended by dr Katarzyna Stepniowska from Department of Swine Diseases and MSc Aneta Pluta from Department of Biochemistry of NVRI, Pulawy who took part in the course: "qPCR training - High throughput qPCR" in Copenhagen, Denmark from 11th to 18th April 2016. The participants were particularly interested in comparing the gene expression analysis techniques and improving their skills. The senior researcher from the section for immunology and vaccinology, dr Kerstin Skovgaard was the head of the project which included experimental design, RNA extraction and quality control, cDNA synthesis and pre-amplification, high throughput microfluidic qPCR (BioMark) and data preprocessing with analysis using GenEx software.



Pic.1. The attendees and training staff of short term mission at the DTU-Vet. From the right: Karen Tarp and Kertstin Skovgaard (DTU, Copenhagen), Aneta Pluta, Katarzyna Stepniewska (NVRI, Pulawy).

On-site training at the NVRI

Information gained during the training, held within the project Vet-Twin on 11th - 18th April 2016 at the DTU, Copenhagen, has been communicated to workers of NVRI as a presentation entitled: "Evaluation of gene expression measurement using high throughput real time PCR". The presentations were given by dr Katarzyna Stepniewska from the Department of Swine Diseases and MSc Aneta Pluta from the Department of Biochemistry of NVRI. The meeting was concerned on measurement of regulatory genes expression responsible for inflammatory processes using the high throughput PCR. The

material used for demonstration was lymph node tissue and bone marrow taken from pigs infected with *Staphylococcus aureus*. During the presentation the following steps have been discussed: collection of tissue samples, tissue homogenization and RNA extraction, RNA quantity and purity, RNA quality, cDNA synthesis, pre-amplification, high throughput PCR and biostatistical analysis of the results. The gene fragments were amplified using 48:48 chip (48 samples tested using 48 primer pairs) by BioMark software. The last step of the experiment was the analysis of data using GenEx software.

The next training in the frame of Vet-Twin consortium was organized at the National Veterinary Research Institute in Pulawy on January 24th- 25th of 2017. Sixty participants were trained by Danish experts: dr Charlotte Kristiane Hjulsgaard and dr Jesper Schak Krog with the help of recently trained scientists from National Veterinary Research Institute: dr Orłowska, dr Niczytoruk and dr Czyżewska-Dors. On the first day, the training was theoretical and included development, optimization and validation of quantitative multiplex PCR. On the second day of workshop trainees experienced practical skills like primers and probes design including LNA, T_m calculation and analysis of primer dimers using CLC Workbench software and the tools available on-line. The workshops were highly valuable and

allowed to improve the theoretical and practical skills concerning the development and optimization of real-time qPCR. Very important aspect of the training was to get acquainted with

Vet-Twin project Work Package leaders and representatives of Partner Institutes met in Pulawy for the second time

On 27th February 2017 representatives of Vet-Twin project (No 692131) partners met at the annual Project Assembly meeting at the National Veterinary Research Institute in Pulawy (NVRI). NVRI was represented by project coordinator – Mirosław P. Polak, BfR by Karsten Noeckler and DTU by Kristian Moller. Project coordinator invited also WP Leaders to present the current status of each WP to the PA members. The meeting was focused on discussion regarding progress done within the particular WPs for the first year of Vet-Twin duration. The Polish side was represented also by prof. Jacek Kuźmak PhD, ScD (Deputy Director for Research, manager of WP2), MSc Monika Banaszek-Urban, Msc Agata Białek (task managers of WP1), Malgorzata Olejnik PhD (manager of WP4) Dariusz Wasyl PhD, ScD (manager of WP3), Marek Kukier PhD and Grzegorz Woźniakowski PhD, ScD (manager of WP6).

lab work organization, to get some practical laboratory tricks and to understand the scope, usability and functionality of the DTU-Vet laboratory layout.



Pic. 2. Presentation of the most urgent deliverables of Vet-Twin Project by project coordinator.

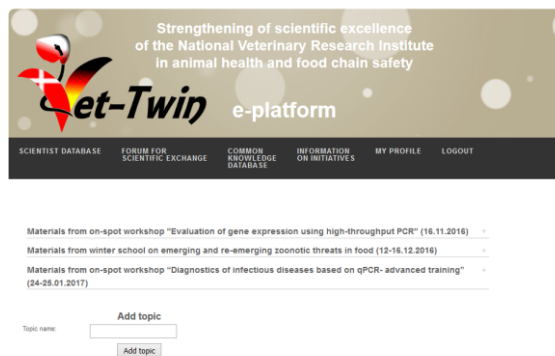
During the first year of realization, project was well managed. In March 2016 project coordinator together with project assistant, participated in a ‘Coordinators Day’ in Brussels. They had the possibility to meet Monica Hoek, who at that time was Vet-Twin Project Officer (PO). Then the PO was changed and now it is Soraya Impens.



Pic. 3. Mirosław Polak, PhD ScD (project coordinator) during preparation of the proposal for organisation of summer school in 2017.

E-Platform (WP3) has been prepared and is operational, but only NVRI scientists are registered on it. The purpose of this platform is to use it for

direct communication among researchers from partner institutions. Therefore the scientists from BfR and DTU should register there as soon as possible. The established forum site enables discussion on different scientific topics as well as experience exchange. It is also meant to help find the partners in common research areas and to prepare grant proposals.



Pic. 4. Vet-Twin website with e-platform for members registration and knowledge exchange.

Almost all trainings (within WP4) for NVRI researchers at BfR and DTU have been organized.

Within Vet-Twin there is a possibility of participation in conferences to present the project and its participants within WP6. A roll-up about Vet-Twin project has been prepared to promote the project during thematic annual conferences at NVRI.

Due to the establishing of the new Department of Omics Analysis at the NVRI directed by Dariusz Wasyl PhD, ScD it might be possible to enrich the trainings for PhD students regarding application of novel diagnostic molecular biology techniques, especially in terms of application and analysis of next generation sequencing (NGS).

During the PA meeting representatives of each partner also had the occasion to discuss about the sources of funding and about the strategic planning in each institution.

To summarise, the meeting was a successful event in terms of clear identification of next steps to be taken, especially having in mind the transfer of DTU-Vet staff and equipment to a new facility on the outskirts of Copenhagen.

Report from Vet-Twin Winter School

The first winter school of Vet-Twin was focused on emerging and re-emerging zoonotic threats in food and was held in Pulawy from 12th to 16th December 2016.

The winter school was attended by 18 people, 14 of scientists from National Veterinary Research Institute (Pulawy, Poland) and 4 attendees from Ukraine (2 scientists from State Scientific-Research Control Institute of Veterinary Medicinal Products and Feed Additives, Lvov and 2 scientists from Institute of Experimental and Clinical Veterinary Medicine, Kharkov). During five days, a total of 13 interactive lectures were presented combined with hands-on exercises.

In the first part of winter school, three lectures given by Dr. Anne Mayer-Scholl (BfR Berlin, Germany) were focused on microbiological risk assessment (MRA) and involved the introduction to basic concepts and definitions as hazard identification and its characteristics, risk assessment, or evaluation of data quality. The theoretical introduction on MRA was followed by a lecture on trichinellosis and was given by Dr. Annette Johne (BfR

Berlin, Germany). The lecturer presented all information about *Trichinella spp* i.e. its characteristics, transmission, distribution, host adaptation, pathogenesis, clinical manifestations, gross lesions, diagnosis, prevention and control, incidence, distribution and epidemiology. During the sessions the participants worked interactively in groups with an objective to solving specific tasks, e.g. assessing the risk of human *Trichinella* infection through pork and pork products.

In the second part of winter school (with Łukasz Bocian from NVRI Pulawy, Poland as the lecturer) attendees were introduced into the aspects related to methods of mathematical modeling that can be used for a starting point for quantitative risk assessment. They were familiarized with basic concepts of the probability distribution, iteration, Monte Carlo simulation and principles for sample size calculations.

Next, five presentations given by Dr. Dariusz Wasyl (NVRI) were focused on antimicrobial resistance (AMR) beginning from basic concepts as methods for resistance determination, criteria for results interpretation, resistance monitoring in Poland and the EU, resistance to specific antibiotics of *Salmonella* and *E.coli* serovars isolated from domestic animals but also from wildlife.

Three presentations of Dr Bernd-Alois Tenhagen (BfR Berlin, Germany) also concerned AMR in the food chain (general considerations and potential its importance) but also risk assessment of AMR for human health.

Selected (copyright issues) presentations are available on e-platform.



Pic. 5. Participants of Vet-Twin Winter School, held in Pulawy, 12-16.12.2016.

Overall the cooperation of Vet-Twin partners during the first year of project realization was good. Since during following years more activities will take place in NVRI, partners from BfR and DTU are expected to participate in them as experts. Therefore they need to have in mind a few visits to NVRI each year. Dates for trainings and winter/summer schools will be consulted with partners well in advance to find dates suitable for all.

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