Final report club 5 joint research

Matrix-assisted laser desorption/ionization time of flight MALDI-TOF, mass spectometry for identification of bacteria in veterinary medicine

Participating partners:
P1. National Veterinary Institute (SVA), Sweden
P2. Central Veterinary Institute of Wageningen University and Research center (CVI), Netherlands
P3. National Veterinary Institute, Technical University of Denmark (DTU.VET), Denmark
P4. Animal Health and Veterinary Laboratory Agency (AHVLA). Weybridge, Surrey, England
P5. The Norwegian Veterinary Institute, (NVI), Norway*, associated partner
P6. Public Health Agency of Sweden (FOHM) former The Swedish Institute for Communicable Disease Control, (SMI) Sweden*, associated partner
*P5 and P6 are not CoVetLab partners, but are keen to be involved and will contribute own money for their part of the project.

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Date started:	October 2013
Length of project	12 month
End of project:	September 2014

Project Team

Names	Institute	Role in project
Erik Eriksson	SVA	Projectleader
Eva Jansson	SVA	Partner, Fishpathogens
Olga Haenen	CVI	Partner, Fishpathogens
Branko Kokotovic'	DTU.VET	Partner
Inger Dalsgaard	DTU.VET	Partner, fish pathogens
Luke Randall	AHVLA	Partner, Brachyspira

Research Questions

Improvement of the MALDI-TOF typing systems ability to identify bacteria of veterinary interest by construction of MSPs for important fish pathogens and bacteria causing diarrheal disease in pigs of the genus *Brachyspira*.

Key Objectives:

To update the database for MALDI-TOF with MSPs of important veterinary pathogens not already included. To evaluate the use of MALDI-TOF and the new updated database with traditional diagnostic techniques for the veterinary pathogens, with own collections of bacteria of participating labs. To evaluate the use of MALDI-TOF for direct identification of bacteria growing in blood culture bottels.

Brief workplan, including key milestones

I. Production of Main Spectra Projection (MSP)s

The fish pathogen part will focus on bacteria belonging to the genera *Aeromonas, Flavobacterium, Yersinia* and *Vibrio.* Partners involved in the fish part will be SVA, CVI and DTU.VET. Division of work for MSP production will be as follows:
SVA: *Aeromonas salmonicida* susp. *salmonicida*, *A. s. achromogenes*, *Flavobacterium columnare, F. psychrophilum*
DTU.VET: *F. psychrophilum, Vibrio anguillarum, Yersinia ruckeri*
CVI: *Vibrio scophthalmi/ichthyoenteri* and *V. vulnificus*
The *Brachyspira* part will produce MSPs from bacteria belonging to the genera *Brachysprira.* Several institutes in the network have a broad strain collection of different *Brachyspira* species which can be used for creation of spectra and evaluation of created spectra.

II. Exchange of bacteria

Isolated bacteria from the participating laboratories will be exchanged and tested by MALDI-TOF on the other laboratories for evaluation of the quality of identification (obtained score).

III. Specificity of identification

A selection of isolated bacteria of other genera than above mentioned, from fish or water and clinical samples will be tested by MALDI-TOF when the local databases have been updated with the new MSPs, to evaluate the specificity of the assay.

IV. Direct identification of bacteria growing in blood culture bottels

SVA will perform evaluation studies with spiked blood samples and spiked synovial samples inoculated in blood culture bottels to assess the MALDI-TOF systems capacity for direct identification in blood culture bottels. Routine clinical samples from horses will be examined in parallel with SVA´s routine methods to evaluate performance of the MALDI- system in combination with MALDI SepsityperTM Kit. SMI, the project leader in the MALDI network for MALDI-TOF work regarding BSL3 agents, is interested to participate in this project, for work on *Brucella*, as a cooperative partner with their own budget

V. Ring trial

A ringtrials will be sent out to all labs in the MALDI-network, including fish pathogens and other veterinary microbes. The results will be collected, compiled and distributed to all participants.

VI. MALDI-TOF workshop

Organization of a MALDI-TOF Workshop at SVA for partners in the CoVetLab and the MALDI-TOF network.

VII. Common website

A website, emanated from the CoVet Lab homepage, will be created for communication in the network. SVA will construct this website and all partners will take active part in updates.

Milestone No	Milestone title	Month
1.	Production of Main Spectra Projection (MSP)s.	Achieved
2.	Exchange of bacteria for evaluation of of the quality of identification	Partly achived
3.	Specificity of identification investigated	Achived
4.	Direct identification of bacteria growing in blood culture bottels evaluated	Achived
5.	Ring trial distributed and evaluated	Achieved

6.	MALDI-TOF workshop organized and held at SVA for partners in CoVetLab and the MALDI-TOF network	Achieved
7.	Common website for CoVetLab partners and also for MALDI-TOF users in the MALDI-TOF network are available at SVA:s web	Achieved

Planned output

The present project have produced MSPs of important veterinary bacterial species for use as a complement to Bruker´s database at CVI, DTU-VET and SVA. Results from the project will be presented at EAFP´s 17th International Conference on Diseases of Fish and Shellfish in Las Palmas de Gran Canaria, Spain in September 2015SVA, DTU-VET and CVI plan to co-write a paper about MALDI-TOF for identification of fish pathogens.

Deliverable No	Deliverable title	
1	Production of Main Spectra Projection (MSP)s for fish pathogens. (SVA, CVI, DTU.VET)	Achieved
2	Production of MSPs for *Brachyspira* spp. (AHVLA, SVA, DTU.VET)	Achieved
3	Distribution of a panel of isolates from each laboratory that has been used for the production of MSPs, to project partners (AHVLA, SVA, CVI, DTU.VET).	Partly achieved*
4	Isolates obtained from partners are tested by MALDI-TOF and spectras are evaluted in each laboratories own database (AHVLA, SVA, CVI, DTU.VET).	Partly achieved*
5	A panel of relevant bacteria from each laboratory are tested by all partners MALDI-TOF for evaluation of specificity (AHVLA, SVA, CVI, VET.DTU).	Partly achieved*
6	Ring trial distributed to CoVetLab partners and partners in the MALDI-TOF network, in total to ten laboratories in Europe	Achieved
7	The MALDI-TOFs system evaluated by spiked samples on fitness for use for direct identification of growing bacteria in blood culture bottels (SVA).	Achieved
8	The MALDI-TOF systems evaluated on genuine clinical samples on fitness for use for direct identification of growing bacteria in blood culture bottels inoculated with clinical samples from animals (SVA).	Achieved
9	The results from the project are summarized in a draft for publication	Partly achived

	* Exchange of MSPs between labs, attached to emails, was a more efficient way to compare results instead of sending bacterial strains between the laboratories.	

Fish pathogens: MSPs have been produced at CVI, DTU-VET and SVA for different subspecies of *Aeromonas salmonicida* (11 MSPs), *Flavobacterium columnare* (8 MSPs), *F. psychrophilum* (16 MSPs), *Yersinia ruckeri* (3 MSPs), *Vibrio anguillarum* (8 MSPs) and one of each of *Vibrio ichthyoenteri*, *V. splendidus* and *V. vulnificus*. Bacterial isolates from the routine bacterial diagnostics and isolates in the labs own collections have been tested by MALDI-TOF and compared with standard techniques for identification as biochemical assays, API or PCR by use of Bruker´s database and the new MSPs. Introduction of MSPs for *F.psychrophilum* and *F. columnare* have been successful and tested isolates have given a good score above 2.0. *F. columnare* grow into the agar medium and it is therefore important to take out the sample for MALDI-TOF identification at an optimal growth status of the bacterium for a good identification. *V. anguillarum* and *Y. ruckeri* are both included in Bruker´s database and the lab´s own isolates have been successfully identified to species level. Several serotypes of *V. anguillarum* and *Y. ruckeri* exsist and also different biotypes of *V. vulnificus* and *Y. ruckeri* with different grade of virulence. So far, differentiation to serotype or biotype for *V. anguillarum, V. vulnificus* or *Y. ruckeri* are not possible with MALDI-TOF and need further developments.The identification of *A. salmonicida* spp. by MALDI-TOF will also need further improvements, as the spectras showed too similar results among subtypes for a correct identification. There are possible techniques available for subtyping in MALDI-TOF and these are recommended for further studies. According to the workplan, bacterial isolates from different labs should be exchanged. We found however that exchange of MSPs between labs, attached to emails, was a more efficient way to compare results instead of sending bacterial strains between the laboratories.

Brachyspira: *Brachyspira* isolates including *B. hyodysenteriae* (n = 4), *B. innocens* (n = 4), *B. intermedia* (n = 8), *B. murdochii* (n = 2), *B. pilosicoli* (n = 7) and *B. suanatina* (n = 2) were sent to AHVLA from SVA and used to create MSPs to be used to facilitate identification of *Brachyspira* by MALDI-TOF. All isolates were grown successfully and improved growth was observed if FABA (Fastidious anaerobic blood) agar if the incubation temperature was increased from 37°C to 40°C. MSPs were created for most of the isolates, and these were evaluated by identification against themselves, identification against the entire Bruker´s database of MSP spectra, identification against an unpublished set of eight Bruker *Brachyspira* MSPs and by creation of dendrograms. On the basis of these results, it would seem acceptable to disseminate selected *B. pilosicoli, B. innocens, B. hyodysenteriae* and *B. murdochii* MSPs to Co-Vet-Laboratories and for inclusion in the Bruker´s database. Present results suggest that these MSPs will not always give reliable results to species level, possibly except for *B. pilosicoli.* Further improvements by production of more MSPs to well characterized *Brachyspira* strains are therefore suggested for a more reliable identification to species level. This should improve the ability of MALDI-TOF to identify *Brachyspira* to the genera and *Brachyspira pilosicoli* to the species.

Ringtrial: A ringtrial (profiency test) was distributed in May 2014 from SVA. In addition to the five CoVetLab institutes, five other institutes participated, EVIRA Finland, FOHM Sweden, Vetsuisse Switzerland,GD The Netherlands and IZVE in Italy. The ringtrial consisted of ten bacterial isolates. The results from the ringtrial were compiled in such a way that it enabled the institutes to compare their results with all the others participants anonymously. All results from the ring trials have been distributed to the participating institutes.

Direct identification of bacteria in cultivated blood agar bottels by MALDI-TOF:

A study was performed with horse synovia spiked at four inoculations levels with three different bacteria *Actinobacillus suislike, Streptococcus equi* subsp*. zooepidemicus* and *Staphylococcus aureus*. Spiked samples were inoculated in blood agar bottels (SVA´s own production). Broth from the bottels were extracted and examined by direct typing on MALDI-TOF after incubation for 4 h, 6 h, 8 h and 24 h in 37° C. A correct identification was possible for *Actinobacillus suislike* after 4 h incubation in the highest inoculation level. All bacteria were detected after 24 h incubation with direct typing on MALDI-TOF. A similar study was conducted with horse blood inoculated with *Streptococcus equi* subsp*. zooepidemicus, Actinobaccilus equ~~u~~li* and *E. coli* using the Bruker´s MALDI Sepsityper ~~iter~~ kit before extraction. The *Streptococcus* species and *E. coli* were correctly identified at the lowest inoculation level, 10 cfu/ ml blood. *Actinobacillus equ~~u~~li* was correctly identified to genus level. From SVA:s routine diagnostics, 16 clinical samples inoculated in blood culture bottels with suspected growth of bacteria, were analysed by inoculation on agarplates compared with direct MALDI-TOF typing. Successful typing by MALDI-TOF occurred in 14 of the 16 cases.

Workshop: A workshop were held at SVA in Uppsala 12-13 of June 2014. In total 15 international participants and 8 participants from SVA attended the workshop. Participants were present from all CoVetLab institutes except from ANSES, France. Also attending the workshop were representatives from Bruker Daltonics MALDI-TOF Manufacturer, EVIRA Finland, FOHM Sweden, Vetsuisse Switzerland, GD The Netherlands and IZVE Italy. The workshop was hold as a lunch to lunch meeting and included presentations from Bruker Daltonics and presentation on MALDI-TOF projects from the different institutes. Results from the ringtrial was discussed in detail, the new website for the MALDI-TOF network were presented and future cooperation projects were discussed.

Problems experienced

Problems to keep the web page up to date after that the project was finished. Several networks are in operation about MALDI-TOF and it is preferred to gather around one or two networks.
It was difficult to construct good quality spectra from the Brachyspira strains. The results improved somewhat with alterations in how the strains were cultivated but were still not optimal.

Value of cooperation for club 5 institutes
Cooperation in the evaluation of a new technology for identification of veterinary pathogens has been quite useful as several aspects and pathogens have been possible to cover. Practical issues have easily been discussed under in-formal meetings. The ring trial has been important for implementation of MALDI-TOF in the quality assurance for the diagnostic labs. It has been stimulating to participate in a project where pathogens from several different animal species have been represented.
Any other information
The cooperation between particiapting labs and the network continue. A new ring trial will be sent out by GD and a new workshop will be organized by CVI in April 2015. Personal from CVI will visit SVA in March 2015 for exchange of experiences.
Evaluation: The MALDI-TOF project have been very succesful with a big interest also from laboratories outside CoVet Lab that have, after request, particpated with their own budget. The cooperation have made it possibel to cover more pathogenic bacteria for a a cost effcient validation of the technique. This cooperation have also opened up for further collaborations in the future.

Deliverables met?	YES
Start of project	2013-10-01
Cooperation	All partcipants have contributed to a positive and open atmosphar in the project and expertise from several areas have been covered.
Advice towards new calls / new projects	Continue MALDI-TOF coopertion among CoVet Labs as this new technology is expected to dominate the diagnostic work in veterinary bacteriology in the future. The present project have clearly demonstrated the many advantages with the MALDI-TOF technique in veterinary medicine, especially after improvements through the project.The project has identified areas for further improvements of the technique. There are several possibilities to increase the use of MALDI-TOF in new applications, as in subtyping of bacteria and for other kinds of pathogens as virus and parasites.