Diagnosis of Pandemic Influenza A H1N1 virus in Danish pigs

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EAVLD Newsletter

Foreword

This newsletter...

Dear colleague

This is the third Newsletter for members of the EAVLD. The first two Newsletters were sent to members in May and December 2010. They are available on the EAVLD website **www.eavld.org** under Member pages.

In this issue we present some information about Matrix Assisted Laser Desorption / Ionization Time-of-flight (Maldi-TOF) mass spectrometry as a tool for the identification of micro-organisms, diagnosis of pandemic influenza A H1N1 virus in Danish pigs and tumour diagnosis of dogs and cats in relation to veterinary oncology.

At the end of the Newsletter you find a list of the EAVLD board members.

EAVLD NEWS

EAVLD Congress 2012 in Poland

In 2010, the first EAVLD Congress was successfully held in the Netherlands, with more than 250 scientists visiting this congress. The second EAVLD Congress will be held in Poland, in 2012, and will be organized by the National Veterinary Research Institute (NVRI) in Pulawy. The NVRI has experience in hosted congresses and large meetings, showing an excellent organisation talent. More information will become available soon and EAVLD members will be kept informed on the progress of the organisation of this congress.

New website layout

As you may have noticed the appearance of the website has changed. The reason is that we decided to change program to build the website in order to facilitate administration and improve the visual appearance. **NB: Members login can be found in the upper right corner!**

A new feature of the website is the PayPal button that simplifies payment for membership using credit cards. We hope that this will be helpful. Now, as payment options have been improved, new members will have to pay before being allowed into the member pages.

In order to facilitate the interaction between members we have now started a discussion page for members. The members are strongly encouraged to make use of this discussion forum and to come up with proposals for activities on the website. If you experience any problems with the website, please report to Frederik.Widén@sva.se

Matrix Assisted Laser Desorption / Ionization Time-of-flight (Maldi-TOF) Mass Spectrometry and the identification of micro-organisms

Matrix-Assisted Laser Desorption/Ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules (biopolymers such as proteins, peptides and sugars) and large organic molecules (such as polymers, dendrimers and other macromolecules), which tend to be fragile and fragment when ionized by more conventional ionization methods. The ionization is triggered by a laser beam (1). The type of a mass spectrometer most widely used with MALDI is the TOF (Time-Of-Flight mass spectrometer), mainly due to its large mass range.

MALDI-(TOF) is used in biochemistry, organic chemistry, polymer chemistry but also in microbiology. MALDI-TOF spectra can be used for the identification of micro-organisms such as bacteria or fungi. Species diagnosis by this procedure is much faster, more accurate and cheaper than other procedures based on immunological or biochemical tests (3).

Identification of micro-organisms by Maldi-TOF

Fast and reliable identification of micro-organisms is a crucial step toward an appropriate treatment of infectious diseases in medical and veterinary diagnostics and is of main interest today. Mass spectral identification of micro-organisms has been shown as a tool for rapid identification. Identification of micro-organisms by MALDI-TOF-MS can be applied to a wide array of bacteria, yeasts, and moulds. A small amount of cells from a plate is sufficient for an automated and rapid identification (2, 3).

Procedure for the identification of micro-organisms

The sample preparation is very simple and easy to perform. After selection of the colony of interest, the colony is removed from the culture plate and dissolved in acetonitrile. A small volume is placed on a steel target surface and dried by air. After drying, the steel target surface is placed in the Maldi-TOF mass spectrometer, and mass-spectra are collected. The mass-spectra of the unknown micro-organism will be compared with known bacterial reference strains in the database (Figure 1).

Conclusion

MALDI TOF-MS is a valuable tool in biosciences for obtaining accurate mass determinations, primary sequence information, and it can also be used or the identification of micro-organisms. Validation studies have shown that the species diagnosis by MALDI-TOF is much faster, more accurate and cheaper than other procedures based on immunological or biochemical tests. Therefore, MALDI-TOF may or will become the standard method for species identification in medical microbiological laboratories over the next few years. However, it is of great importance that the MALDI-TOF database contains as much as possible bacterial reference strains, not only from human origin but also from veterinary origin. Therefore, co-operation is needed between the different labs to enlarge the data-

To be continued on next page..

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base for the identification of micro-organisms from veterinary origin.

If institutes/laboratories are interested in co-operation, please contact;
g.wellenberg@gddeventer.com

References

1: http://en.wikipedia.org/wiki/Matrix-assisted_laser_desorption/ionization

2: Alispahic M., et al., VetOMICS, 2008.

3: Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D. 2009. "Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry." Clin Infect Dis. 49 (4): 552–3.

Gerard Wellenberg

GD-Deventer

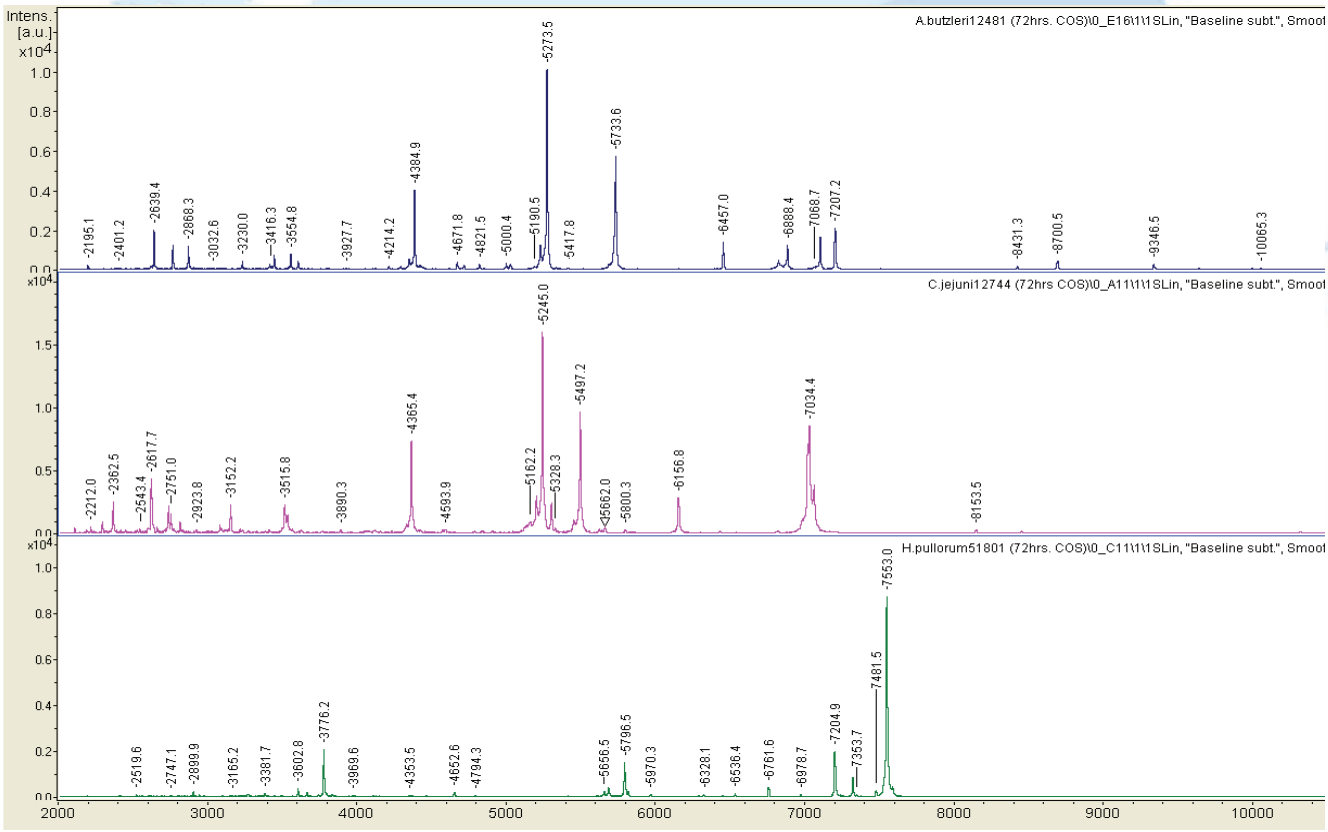


Figure 1:

Spectra of Arcobacter butzleri, Campylobacter jejuni, and Helicobacter pullorum (Figure presented by reference 2).

Diagnosis of Pandemic Influenza A H1N1 virus in Danish pigs

Influenza subtypes H1N1, H3N2 and H1N2 are circulating in pig populations worldwide. In March-April 2009, a novel pandemic Influenza A H1N1 virus (H1N1-2009v) of swine origin emerged in the human population globally and subsequently has also been diagnosed in pigs from several countries. The H1N1-2009v virus differs genetically from the swine H1N1 viruses that have been circulating in pigs worldwide for decades, thus diagnostic assays for swine influenza virus needed validation to assure detection of the new H1N1-2009v virus.

At the National Veterinary Institute in Denmark, detection of swine influenza virus in clinical specimens is routinely performed by real-time RT-PCR targeting the M or NP genes. These assays have been validated for detection of the H1N1-2009v virus by *in silico* analysis of primer and probe target regions and by testing known positive samples of human origin. Thus, it has been confirmed that these assays also detect the new variant virus, and are unable to discriminate between the previously circulating strains and the H1N1-2009v variant.

For specific detection of the H1N1-2009v subtype, a real-time RT-PCR assay targeting specifically the HA gene of the H1N1-2009v virus that was developed for human diagnosis of H1N1-2009v at the State Serum Institute, was validated on archived influenza virus positive swine samples. The assay turned out to be specific for the H1N1-2009v subtype only.

Since 2009, the diagnostic strategy at the national Veterinary Institute has been to routinely subtype all samples that are positive for swine influenza virus by M and/or NP real-time RT-PCR, with the H1N1-2009v specific assay. The first case of H1N1-2009v in a Danish pig herd was diagnosed in January 2010, and since then approximately 10 % of the swine influenza virus positive herds are of this subtype. Thus, the H1N1-2009v virus appears to have become established in the Danish pig population together with the previously recognized swine influenza subtypes.

By Charlotte K Hjulsager, National Veterinary Institute, Technical University of Denmark.

YEAR	SIV positive herds	H1N1-2009v positive herds
2009	81	0
2010	96	9
2011*	47	10

*Number of Danish herds diagnosed positive for swine influenza virus (SIV) and H1N1-2009v at the National Veterinary Institute, Technical University of Denmark until May 1st 2011**

Tumour diagnosis of dogs and cats: The new area of veterinary oncology

The number of dogs and cats in each country is high. For example in the UK, there are 6.8 million dogs and 9.6 million cats, and in The Netherlands 2.1 million dogs and 3.6 million cats.

Dogs and cats are becoming more and more family members. Their living standard and consequently their lifetime have increased the past decade. Due to a longer lifetime, more and more dogs and cats will develop tumours. Nowadays, one of each three dogs and cats in The Netherlands will develop cancer, which means more than 100.000 new cases every year.

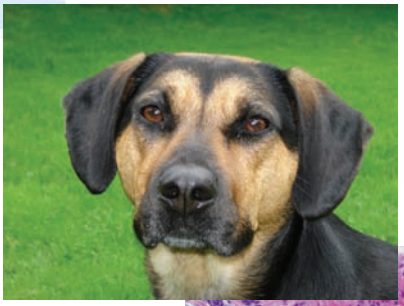
Owners of dogs and cats ask for more extensive diagnostic investigations concerning all kind of diseases. This includes more specific tumour diagnosis in order to find the best treatment for their pet. With the right diagnosis, veterinary practitioners can start a discussion, together with the owners, how to treat the tumour.

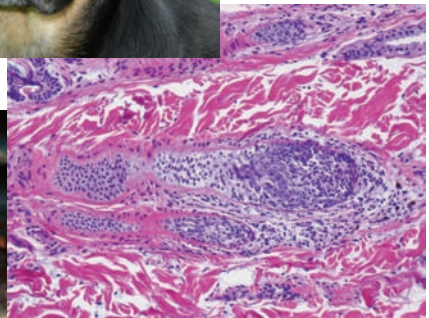
New opportunities for treatment also ask for higher standards of pathological investigations. Pathological investigations, based on microscopic parameters, can give answers on the type of tumour, e.g. benign or malignant, and it can open new ways for treatment. Based on the right diagnosis, the vet can make more strategic choices for treatment.

In well equipped and occupied veterinary oncology labs, different kind of materials and samples can be investigated to search for the right diagnosis concerning tumours and skin lesions. Cytological, immunocytological and histopathogical investigations are widely used within the veterinary oncology laboratory, and new techniques are introduced to improve the quality. Technological innovations, such as the introduction of DNA techniques, and the use of the special staining techniques, KI 67 and Ag-NOR, have found their ways within our laboratory. Staining techniques in the immunocytopathology can be used for the identification of malignant lymphoma's; to investigate the expression of CD3 or CD79a by these lymphoma's. Based on these techniques it is possible to examine whether we have to deal with a T-cell of B-cell lymphoma.

As you will understand, the field of veterinary oncology is moving quickly forward. It follows the new developments within the human oncology like a shadow. This new area is improving more and more as owners of dogs and cats would like to give their pets the best possible treatment.

Gerard Wellenberg
GD-Deventer





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- Jose Antonio Garcia, Universidad Complutense, Spain
- Gerard Wellenberg, Dutch Animal Health Service, The Netherlands

Gerard Wellenberg was elected as a new member of the board at the general meeting in the Netherlands in September 2010.

Short presentation

Gerard Wellenberg was born on July 20, 1958. Gerard studied Medical Microbiology in Deventer, The Netherlands. He started his active career at the Regional Medical Health Laboratory and the Regional Animal Health Services in 1977 and 1980, respectively. During this period, he studied Immunology and Biochemistry at the Open University in The Netherlands. In the period 1992 – 1994, he studied Medical Biology at the University of Groningen. In the next two years, he was setting up an ELISA laboratory at Pharma-Bio Research, a Clinical Pharmaceutical and Biomedical Research centre for the registration of pharmaceutical compounds at the FDA.

He became head of one of the laboratory units within the Virology Department at ID-DLO (Lelystad) in 1996. Gerard fulfilled his PhD study on the role of viruses in the aetiology of bovine mastitis in 2002.

From 2005 on, he is senior scientist in Molecular Biology and Virology at the Dutch Animal Health Service in Deventer. Since 2001, he is also active as veterinary consultant in national and international projects.

You can read short presentations of the other board members in the first Newsletter which you can find on the homepage: http://www.eavld.org/attachments/Newsletter/EAVLD_Newsletter_1_2010-04-19.pdf (NB logon for members is needed)