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EAVLD Newsletter

This newsletter...

Foreword

Dear colleague,

In this seventh issue of EAVLD Newsletter you will find information about the WAVLD Congress 2013 and the next EAVLD Congress 2014. We include two scientific abstracts: The first about a new method for safe submission of FMD-positive material to the diagnostic lab and the second about diagnostics of Schmallenberg virus In Poland. Finally, there is some information about the membership database on the EAVLD homepage and the present EAVLD board members.

Congress announcements

EAVLD Congress 2014

We are happy to announce the city of Pisa (Tuscany) for the 2014 EAVLD Congress which will take place in mid-October.

Pisa is known worldwide for its leaning tower and is a beautiful historical city. Has its own, well connected international airport and is at 1 hour drive from Florence, where there is also the Peretola Airport.

The venue will be the city Congress Palace (Palazzo dei Congressi, Via G. Matteotti 1 www.palazzodeicongressi.pisa.it), with the possibility to have a main plenary room with up to 500 seats and a second room for possible parallel sessions with 200 seats. MV Congressi has prepared a draft planning and budget and has verified that we can manage to organize the event keeping the registration fees around the 2012 figures, including a social event ("Tuscan Night, to be held within the congress venue).

WAVLD Congress 2013

The 16th International Symposium of the World Association of Veterinary Laboratory Diagnosticians will take place in Berlin June 5-8 2013.

See http://www.wavld2013-berlin.com for preliminary program, venue, registration etc. or check the flyer: http://www.wavld2013-berlin.com/fileadmin/www_wavld2013_de/Home/PDF/12-09-25-WAVLD-Flyer.pdf

Rescue of infectious Foot-and-Mouth Disease Viruses from preserved viral RNA

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Introduction

Foot-and-mouth disease (FMD) remains one of the most economically important diseases of farm animals globally. It is caused by FMD virus (FMDV) which can infect a wide range of cloven-hoofed animals including cattle, pigs and sheep plus about 70 wild life species, e.g. buffalo. The disease is widespread especially in Africa and Asia. Although Europe is normally free of the disease now, large numbers of outbreaks have occurred close to its boundaries, e.g. within Turkey, and hence there is a threat to neighboring countries. Indeed, Bulgaria experienced outbreaks of disease in 2011 due to a FMDV strain which was most closely related to viruses that had been detected within Turkey. In addition, there are occasional unexplained introductions, e.g. to the United Kingdom in 2001 (which then spread to Ireland, France and the Netherlands) and this resulted in very high economic losses due to loss of trade and slaughter of several million animals.

FMDV is the prototypic *Aphthovirus* within the family *Picornaviridae*. FMDV particles comprise a single copy of the positive-sense RNA genome (ca. 8400 nt in length) within a near spherical protein shell (or capsid). The viral RNA is sufficient to initiate replication when introduced into cells without any requirement for viral proteins. Thus the capsid serves to protect the RNA when the virus is outside of cells and to facilitate delivery of the genome into the cytoplasm of cells.

FMD can be controlled successfully by vaccination. However, there are seven distinct serotypes (O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3) and many subtypes have also been described. Vaccination against one serotype produces little or no protection against other serotypes.

In countries lacking the infrastructure to identify rapidly the serotype of FMDV in samples, these can be transported to reference laboratories or similar facilities, e.g. the World Reference Laboratory at Pirbright, U.K. or DTU-Vet in Denmark, for characterization. However, this has required the transportation of samples containing infectious FMDV which represents a significant bio-security hazard; it is consequently expensive and often slow. Moreover, the virus samples have to be collected and stored under appropriate conditions, until submission, to allow virus to be isolated successfully in the receiving laboratories. In practice, this can be difficult when ambient temperatures are high and the distances from points of collection to national laboratories are large and the in-

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frastructure is poor. It is therefore desirable to have a system where samples from cases of FMD can be treated at the point of collection to inactivate the virus infectivity but preserve the RNA. To perform the complete range of virus characterization procedures it is then necessary to recover infectious virus from the RNA within a contained laboratory environment. Sequence analysis alone is not sufficient to determine the biological characteristics of the virus and the ability of specific vaccines to provide immunity against it.

The availability of commercial reagents which preserve the viral RNA, in conjunction with systems for the efficient rescue of infectious picornaviruses from RNA, has permitted the development of procedures for the recovery of infectious FMDV from chemically treated clinical samples that contain viral RNA. It has now been shown that FMD viruses can be obtained from inactivated and preserved virus samples that had been collected as clinical samples in Pakistan and Afghanistan.

Rescue of infectious virus from preserved viral RNA

Epithelium tissue samples, from clinically diseased animals, were preserved in *RNAlater* prior to transportation to DTU-Vet, Lindholm. This reagent inactivates RNA degrading activity (RNAse) and it also has a relatively low pH (5.2) which will inactivate the very acid-labile FMDV. From these preserved and inactivated samples, RNA was isolated and the level of FMDV RNA was quantified by specific real time RT -PCR assays. RNA samples containing a significant level of FMDV RNA were introduced into cells using electroporation (essentially producing transient "holes" in the cell membrane by an electric shock) to "rescue" infectious virus.

Once the FMDV RNA is inside susceptible cells then it is able to initiate its normal infectious cycle. The RNA is translated to make the virus proteins required to replicate the RNA and to assemble new infectious virus particles which can then be grown further in cells.

This procedure has been successfully applied to samples of different FMDV serotypes. The rescued viruses can also be used in virus neutralization assays (VNT) which are important for determining the suitability of available vaccine strains. The overall scheme is summarized in Figure 1.

In addition, some of the "rescued" viruses have been used in challenge experiments within cattle housed within a high containment facility. Two viruses, a serotype O and a serotype Asia-1 strain, were inoculated, into calves.

Typical signs of FMD, including elevated

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temperature and vesicular lesions on the feet and in, and around, the mouth were observed in each case and the disease spread to in-contact animals. Thus the "rescued" viruses retained the pathogenicity associated with this virus.

Conclusion

It is believed that the procedure described here should facilitate the characterization of FMDVs circulating in countries where the disease is currently endemic and thus enhance disease control globally.

A more detailed description of this work has been published:

Belsham GJ, Jamal SM, Tjørnehøj K, Bøtner A. Rescue of foot-and-mouth disease viruses that are pathogenic for cattle from preserved viral RNA samples. PLoS One. 2011 6(1):e14621. doi: 10.1371/

Fig. 1.

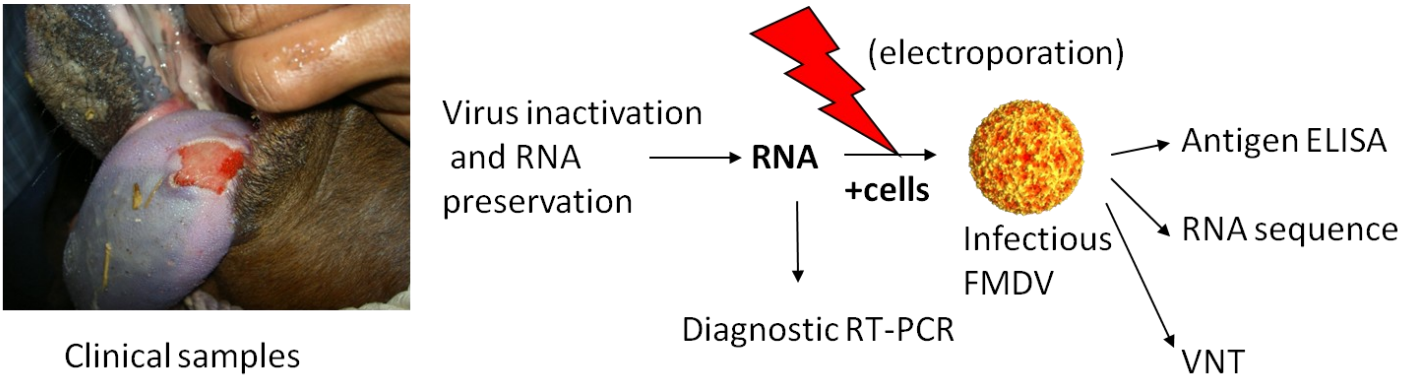

Figure 1. Outline of virus "rescue" from RNA extracted from clinical samples

Schmallenberg virus in Poland and the use of IPX method for virus identification in cell culture

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Since its emergence in Germany in October, 2011, a novel virus of ruminants called the Schmallenberg virus (SBV) has spread to several European countries. Poland so far was regarded as SBV-free based on limited serological and virological studies of native-born cattle and sheep including aborted fetuses and calves born with malformations. Additionally, the analysis of pooled samples of midges collected for BTV monitoring from different regions of Poland was also negative in studies done at NVRI.

Two outbreaks of Schmallenberg virus (SBV) infection occurred in West Pomerania and Silesia provinces of Poland, after two bulls imported from France (FR1 and FR2) were introduced into two herds located in those regions in August 2012 (Fig. 1).

Fig. 1. Geographic location of two SBV-positive herds (FR1 and FR2).

Transmission of SBV into Polish cattle herds where the French bulls were imported was confirmed by viral RNA detection in real-time RT-PCR, virus isolation followed by immunoperoxidase staining and seroconversion detected by commercial ELISA test. Viral RNA was detected also in *Culicoides obsoletus* pools caught in the trap located 5 km from FR1 farm in September 2012 but not in midge pools from the same source tested in October 2011. While real-time RT-PCR relied on the protocol provided by Dr. Martin Beer and Dr. Bernd Hoffmann from FLI, Germany, who also kindly supplied us with positive RNA samples, the immunoperoxidase test (IPX) following virus isolation in BHK-21 cell line was a newly developed in-house technique. More detailed description of IPX is given below.

SBV isolation test was carried out for all real-time RT-PCR positive samples. In brief, 50µl of each sample was placed in duplicate in 24-well microplate to adsorb for one hour at 37°C on approx. 80% confluent BHK-21 clone 13 monolayer. Plates were then overlaid with GMEM supplemented with antibiotics and incubated at 37°C in 5% CO_2 atmosphere for 4-5 days. It was repeated twice (two blind passages)

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with the last incubation shortened to 48-72 h. Following fixation with 20% acetone, the cells were incubated with SBV antiserum collected from FR1 bull in 1:10 dilution for 1 h and subsequently with rabbit anti-cow serum conjugated with horseradish peroxidase. After adding a solution of 3-amino-9-ethylcarbazole (AEC) and HRP substrate, the plates were examined microscopically for the presence of virus specific staining. Uninfected BHK-21 cells served as the negative control for IPX staining (Fig. 2).

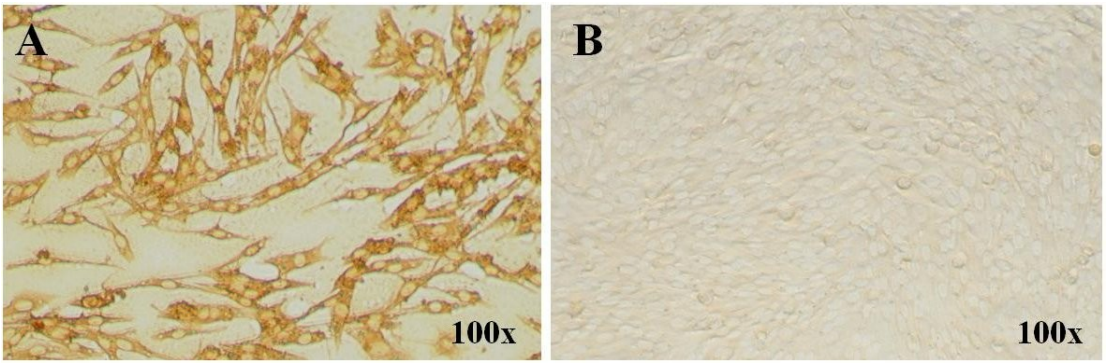

Fig. 2. IPX staining of SBV positive (A) and mock-inoculated BHK-21 monolayer cells (B).

New membership database

To simplify contact between members and the administration of membership a new membership database has been launched on the EAVLD homepage www@eavld.com. To reach the members database you have to log in and click on the button at the top of the page. Through this database you have the possibility to contact other members if they have not blocked this option. Please note that this database is not yet complete as some member's information remains to be transferred. So, don't worry if you don't find yourself there. The work with making the database complete is in progress.

Next to the database button you can find the buttons "my details" and "my profile". The first one allows you to change some of the information given in the membership application while the second allows you to change log-in details for your account. However, you cannot change your name in "my details" unless you contact the secretary.

The membership application form has also changed and is now on-line and can be reached through the public page. New applicants fill in the form and submit. New members are subsequently guided to the pay-pal payment button. Other payment options are also available. After completing the payment the application is reviewed by the secretary and the treasurer. If everything is in order the full membership will be granted and log-in details sent out.

New board members elected at the general meeting in Poland 2012

Lars Ole Andresen was born in 1961 in Ames, Iowa, USA, and graduated as M.Sc. from the Technical University of Denmark in 1987. While employed at the Veterinary Division of the pharmaceutical company Nordisk Droge A/S Lars Ole finished his post graduate education as Industrial Researcher (~PhD) in 1990. Since 1990 Lars Ole has been employed at the institution that today is the National Veterinary Institute in Denmark. His research has comprised development of ELISAs and PCR for research, surveillance and diagnosis of infectious diseases, characterization of disease causing bacterial agents, antigens and virulence factors, including development of detection and purification procedures for proteins and lipopolysaccharide, molecular cloning and preparation and purification of recombinant proteins.

Further, Lars Ole performed studies on pathogenesis and development of infection models in pigs. Vaccinology has also been a key research activity that included development of experimental vaccines, subunit vaccines and autogenous vaccines, against pleuropneumonia, edema disease and exudative epidermitis in pigs, as well as evaluation of vaccines and therapeutic antisera in experimental animal models and in clinical field trials. Current employment as Senior Advisor comprise supervising routine diagnostic methods, approving and interpreting results of laboratory analyses and advising veterinarians. Lars Ole was elected as member of the EAVLD board in 2012

Miroslaw Polak was born in 1964 in Pulawy (Poland), is married and has two daughters (Zuza and Gabi). He studied Veterinary Medicine in Lublin, where he graduated in 1990. Between 1990 and 1992 he worked at the Animal Hygiene Department of the University of Agriculture in Lublin, where he had classes with students and worked on modern breeding systems for pigs with special emphasis on welfare issues of sows and newborn piglets.

In 1992 he started working at the National Veterinary Research Institute in Pulawy at the Department of Virology. In 1994 he was awarded Fulbright Fellowship at the University of Nebraska, Lincoln, U.S., where he spent 13 months doing research on BVDV thermosensitive mutants and learning modern molecular biology based laboratory methods. In 1998 he did his PhD on BVDV including experimental inoculation in calves and isolation of field isolates in cell culture followed by molecular biology typing. In 2010 Miroslaw was awarded the habilitation degree (associate professor) based on his work on BSE in Polish cattle.

In 2012 he was involved in the preparation of the second EAVLD congress, held in Kazimierz Dolny, July 1-4, 2012, where he was elected the second vice-president of the EAVLD.

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Gian Luca Autorino was born on March 24, 1956. Gian Luca studied Veterinary Medicine in Perugia and graduated in February 1980. He received a Master in Management of Health Institutions at the LUISS University in 1997. Since 1984 Gian Luca held a position at the Instituto Zooprofilattico Sperimentale of Lazio and Toscana where, from 1995 to the present, is head of the Virology Unit and from 1999 to the present is responsible for the National Reference Centre for Equine Diseases. His major expertise and research are in classical virology and in prevention and control of animal viral diseases and zoonoses.

With particular reference to present project proposal on Equine Infectious Anaemia dr. Gian Luca Autorino is project leader of a funded research programme on "Evaluation of new diagnostic screening protocol applied to the national surveillance programme and verification of the concordance among the methods available; Study of the principal risk factors investigated in independent geographical clusters of infection; Clinical, immunological and virological evaluations of naturally infected animals".
Dr. Gian Luca Autorino is member of the European Society for Veterinary Virology, 2005-2007 member of the board of the Italian Society of Veterinary Diagnostics (SIDILV), and from 2008 to 2011 President of the same association. He became a member of the EAVLD board in 2012.

Kirsty Line obtained a BSc in Biochemistry from the University of Wales, Aberystwyth in 2000, and a PhD in protein structure and function from University of Exeter in 2004.

She then undertook research and teaching in a number of protein structure and function areas, including the investigation of proteins from helminth parasites, including Haemonchus contortus, and Fasciola hepatica.

Kirsty joined Animal Health and Veterinary Laboratories Agency in 2009, taking on the role of Work Group Leader in the regional laboratory Starcross, in the South West of England.

In 2012 she was given the opportunity to take on the role of Laboratory Resource Manager, being part of the Laboratory Services Department management team, and taking on responsibility for two laboratories in the Midlands region of the UK. Whilst at AHVLA Kirsty has been involved in diagnostic test development for TB culture and molecular assays, MAP molecular assays, Campylobacter fetus speciation assay development.

She has also been involved in assessment of MALDI-ToF for bacterial identification, and LAMP assay development, and development of computer LIMS systems to aid laboratory reporting and data collection. Kirsty Line was elected as EAVLD board member in 2012.

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Board members elected at the general meeting in Holland 2010

Willie Loeffen was born on July 24, 1964 in Oss (the Netherlands). Willie studied Veterinary Medicine in Utrecht, graduating in 1991. Between 1991 and 2001 he worked at the Animal Health Service in Boxtel, carrying out field research on Aujeszky's disease, respiratory problems in swine in general and swine influenza more specifically. He also supported swine veterinarians in the field, with respiratory diseases as his main expertise.

In 2001 he started working at the Central Veterinary Institute in Lelystad as project leader on classical swine fever. There he also finished his PhD on swine influenza, which he originally started at the Animal Health Service. Later on he also became project leader for African swine fever and Aujeszky's disease.

He remained interested in swine influenza, being part of the ESNIP European projects on swine influenza, working on avian influenza in swine during and shortly after the AI outbreak in the Netherlands (2003), and recently working on pandemic (2009) H1N1 in swine.

He was the first secretary of EAVLD from 2009 and organised the first EAVLD congress in Lelystad 2010. Willie was elected as the second president of EAVLD in 2012.

Jose A. Garcia qualified from the Veterinary Faculty (Universidad Complutense) in Madrid in 1987. Just as he finished he joined the Animal Health Department in the same Faculty where he has been working since then.

There he also carried out his PhD thesis on different aspects of the pathogenesis of listeriosis. He also worked in the diagnostic service of the Department from the beginning, mainly bacterial diseases of domestic animals, but has also been involved in different cases of wild animals. It was working for this service as he started to work in fish diseases, field in which he has been mainly involved since then.

After his PhD, he spent one year at the Royal Veterinary and Agricultural University in Copenhagen (Denmark), and later six months at the FRS Marine Laboratory in Aberdeen (Scotland, UK).

In 2002 he got a permanent position as Associate Professor in Animal Health in the Veterinary Faculty (Universidad Complutense de Madrid). And he still is involved in the diagnosis of bacterial diseases, now at the Veterinary Clinical Hospital.

He became member of the EAVLD Board in 2009, and was elected at the first General Assembly in Lelystad 2010.

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Frederik Widén was born in 1958, graduated from Vet school in 1986 and spent a few years in large animal practice before starting at SVA (the National Veterinary Institute) in Uppsala, Sweden. At SVA he started at the pathology department before moving to bacteriology and finally to virology. The PhD work, concerning Porcine Cytomegalovirus and supervised by Dr. Malcolm Banks, was mostly carried out in England where he lived together with his family for three years. After the PhD his work has been dedicated to research on Classical Swine Fever and Hepatitis E virus but also to West Nile Fever and emerging viral infections. He has always, since starting at SVA, been involved in routine diagnostic work on a regular basis. He has been treasurer of the EAVLD since 2009.

Martin Beer was born on 19th of August 1966 in Erlangen, is married and has two children (Julius and Nils). He studied veterinary medicine at the Veterinary Faculty of the Ludwig-Maximilians-University in Munich, Germany. He received a DVM degree and finished his thesis about the role of T-cell immunity in BVDV infection at the same university. He held a position at the Institute for Medical Microbiology in Munich, where he was awarded the 'Habilitation', shortly after moving to the Friedrich-Loeffler-Institut (FLI) as head of the National Reference Laboratory for Bovine Herpesvirus type 1.
Martin Beer is director of the Institute of Diagnostic Virology at the FLI, Insel Riems since 2004, and within his institute many of the German National Reference Laboratories, e.g. for FMD, CSF and AI are situated. Martin Beer has been working with several animal viruses like Pestiviruses, Bovine Herpesvirus Type 1, Avian Influenza Virus and Bluetongue Virus with special emphasis on the development of novel vaccines and modern diagnostic systems.

Sven Erik Jorsal was born in 1950 in southern Jutland, Denmark. He is married to Jytte (also a vet.) and they have three children and one grandchild. Sven Erik studied Veterinary Medicine in Copenhagen and graduated in 1976.

He worked as veterinary practitioner for 4 years. From 1980 he worked at the Royal Veterinary and Agricultural University in Copenhagen, where he finished his PhD in 1984. The next 5 years he worked at the Federation of Danish Pig Producers and Slaughterhouses, Laboratories in Roskilde and Kjellerup. From 1989 he has worked at the National Veterinary Institute, Technical University of Denmark (former the Danish Veterinary Laboratory) as senior scientist and veterinary consultant.

The work since 1980 has mainly concerned epidemiology and diagnostics of infectious diseases in production animals and the research topics were primarily diseases of the respiratory and digestive systems of pigs.

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Gerard Wellenberg was born on July 20, 1958. Gerard studied Medical Microbiology in Deventer, The Netherlands.

He started his active carrier 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.

Andrew Soldan qualified from the Royal Veterinary College in London in 1984. After 2 years in mixed practice he undertook an MSc in Tropical Veterinary Medicine at Edinburgh and then worked in Malawi for 4 years helping to set up an epidemiology unit, performing diagnostic work and undertaking research into tick borne diseases. On returning to the UK he worked as a Veterinary Investigation Officer at the Veterinary Laboratories Agency (VLA) Bury St Edmunds lab before going back into mixed practice in Devon. After 3 years in practice he rejoined the VLA as Regional Test Manager for the Southern region of the UK.

During 2001 he was responsible for coordinating various laboratories undertaking FMD serology following the UK outbreak. In late 2001 he was appointed as Programme Manager for International Trade and Head of Laboratory Testing with responsibility for all testing activities at the 16 VLA sites. In 2006 he became the first Commercial Programme manager for the VLA with a role to maximise the value of Intellectual Property generated from VLA research and to grow VLA's work with Industry. In 2009 he was awarded the degree of Doctor of Veterinary Medicine and Surgery by the University of Edinburgh. Since late 2009 he has been Veterinary Director for the VLA. He is married to Brenda (also a vet), has 3 children and lives in Devon. In 2009 he became the first president of the EAVLD.