# 3rd Annual Meeting EPIZONE

"Crossing borders"

12- 15 May 2009

Antalya, Turkey

Hosted by SAP, FMD Institute Ankara

# Programme and Abstracts

EPIZONE

# Content



EPIZONE

# Welcome

# WELCOME

Dear participants,

We welcome you all to the 3rd Annual Meeting of our EU funded Network of Excellence, EPIZONE

*"Crossing Borders"*

The title "Crossing Borders" of this 3rd Annual Meeting relates to our special topic this year, emerging and transboundary diseases, as well as international networking and its extra value, our network's mission and a prerequisite to control epizootic diseases. EPIZONE plays a central role in prevention and control of animal diseases and will contribute to limiting both the risks and damage caused by those diseases in the EU and beyond.

During this meeting you will meet scientists from many different fields of research, but all share their interests in animal disease control. This will give you the chance to share ideas and thoughts, to learn from each other, to build new relationships and to strengthen the existing ones.

EPIZONE has an ambition to expand its Annual Meetings and this year has chosen to offer related projects in the field of Animal Health an opportunity to meet and present their projects. We believe that by combining meetings of EPIZONE and related projects we stimulate interaction and collaboration and will be "crossing borders". You can find all projects at our market/fair together with sponsors and poster presentations.

We would like to thank Dr. Recep Ergül, former director SAP and all involved members of SAP for their generous hospitality, and the great and enthusiastic support in organizing this 3rd Annual Meeting of EPIZONE.

We wish you all a successful and happy meeting

Professor Wim van der Poel, Coordinator EPIZONE
The scientific committee and the organizing committee

**Scientific committee:**

Chair: Professor W. van der Poel (CVI)
Dr Å. Uttenthal (Vet-DTU)
Dr M.F. Le Potier (AFSSA)
Professor F. Conraths (FLI)
Dr L. Powell (VLA)
Dr C. Enøe (Vet-DTU)
Dr P. Gale (VLA)
Dr P. van Rijn (CVI)
Dr E. Erlacher-Vindel (OIE)
Professor A. Donaldson (Bio-Vet Solutions Ltd)
Professor C. Griot (IVI)
Professor M. Czub (University Calgary)
Professor M. Woolhouse (University Edinburgh)

**Organizing committee:**

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Petra van der Laag (CVI)
Margriet Vedder-Rootjes(WUR)
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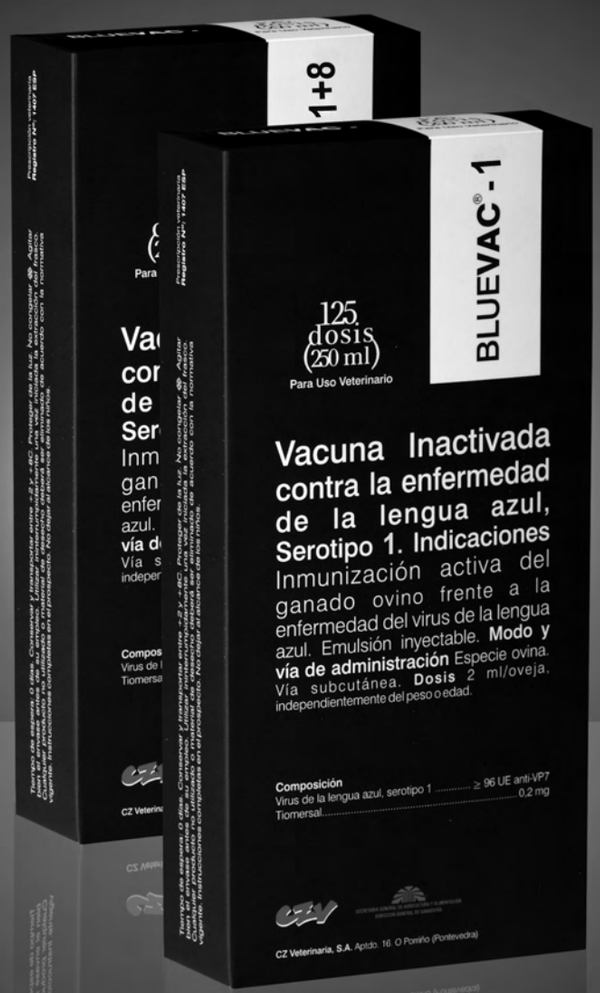


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# Programme

# Tuesday, May 12th 2009

**OPEN SESSIONS**

| | | |
|---|---|---|
| 9.00 – 12.00 | Young EPIZONE<br>\|Room A5\| | |
| 13.00 – 18.30 | Young EPIZONE<br>\|Room A5\| | Theme 4: Diagnostics<br>\|Room A6\| |
| 18.00 – 21.00 | Welcome reception \|Foyer B section\|<br>Registration \|Lobby Hotel\|<br>Poster drop off \|Lobby Hotel\|<br>Presentation drop off \|Lobby Hotel\| | |

**CLOSED SESSIONS***

| | \|Room A7\| | \|Room A8\| | \|Room A9\| | \|Room B6\| | \|Room B9\| | \|Room B10\| | \|Room B11\| |
|---|---|---|---|---|---|---|---|
| 9.00 – 12.00 | WP6.1 | WP6.3 | WP4.2 | WP1.2 | | | |
| 12.30 – 18.30 | WP6.1 | WP6.3 | | | WP7.2 and Theme 7 meeting | | |
| 13.00 – 15.00 | | | | | | WP2.2 | |
| 15.00 – 17.00 | | | | | | WP6.4 | WP6.2 |
| 18.30 – 20.00 | | | | | | EC meeting | |

* *These meetings are only accessible by invitation*

# Wednesday, May 13th 2009

| | | |
|---|---|---|
| 7.30 - 8.30 | Registration | \|Lobby Hotel\| |
| | Poster drop off | \|Lobby Hotel\| |
| | Presentation drop off | \|Lobby Hotel\| |

**8.30 – 9.30** **WELCOME**
\|Hall B1\|

| | |
|---|---|
| 8.30 – 8.50 | *Professor Wim van der Poel, Central Veterinary Institute of Wageningen UR (CVI); Coordinator EPIZONE*<br>'EPIZONE Crossing Borders' |
| 8.50 – 9.00 | *Dr Mehmet Mehdi Eker, Minister of Agriculture and Rural Affairs Turkey*<br>'Welcome' |
| 9.00 – 9.30 | *Dr Francisco Javier Reviriego Gordejo, European Commission, Health & Consumers Directorate-General*<br>'Transboundary and emerging diseases in the context of the new EU Animal Health Strategy' |

**9.30 – 10.00** **KEYNOTE**
\|Hall B1\|

*Professor Manfred Stock, Potsdam Institute for Climate Impact Research (PIK)*
'Predictions, projections, and perspectives of climate change'

**10.00 – 12.00** **ORAL PRESENTATIONS**

| | **Emerging and Transboundary Diseases**<br>\|Hall B1\|<br>**Moderators:** *Dr Piet van Rijn, Dr Paul Gale* | **Theme 4: Diagnostics**<br>\|Hall B3\|<br>**Moderators:** *Dr Åse Uttenthal, Prof Christian Griot* |
|---|---|---|
| 10.00 – 10.15 | *Boone, Ides*<br>Indicators to monitor potential effects of climatic change on animal health: establishing priorities | *Dastjerdi, Akbar*<br>Development of a DNA microarray for simultaneous detection and genotyping of lyssaviruses |

| | | |
|---|---|---|
| 10.15 – 10.30 | *Cetre-Sossah, Catherine*<br>Evidence of recent Rift Valley Fever virus circulation in Mayotte, a French island of the Indian Ocean | *Sareyyupoglu, Beyhan*<br>Development of a Multiplex RT-PCR Technique for differentiation of FMDV serotypes in Turkey |
| **10.30 – 11.00** | **Coffee Break** | |
| 11.00 – 11.15 | *Domingo, Mariano*<br>An experimental infection of European breed sheep with Rift Valley fever virus | *Hoffmann, Bernd*<br>"Double check – double tube strategy" Improved safety for the detection of Bluetongue Virus genome in semen |
| 11.15 – 11.30 | *Moreno, Ana*<br>Diagnostic approach to a West Nile Virus outbreak in Northern Italy on fall 2008 | *Hammoumi, Saliha*<br>Design of a one-step multiplex RT-PCR for the detection and the subtyping of H1, H3, H5, H7, N1, N2 human and avian influenza viruses |
| 11.30 – 11.45 | *Willeberg, Preben*<br>Highly pathogenic avian influenza in wild birds in Denmark 2006: Space and time-space clustering | *Cullinane, Ann*<br>The 2006 Outbreak of Equine Infectious Anaemia in Ireland – New Insights into an Old Disease |
| 11.45 – 12.00 | *Guis, Helene*<br>Mapping the effects of climate change on bluetongue transmission in Europe | *Bergmann, Sven*<br>New strategies in koi herpesvirus (KHV) diagnostics recognizing latent infected carrier fish |

**12.00 – 13.00** **POSTER SESSION / MEET AND GREET AT MARKET / FAIR**

|Hall B2|

- Poster presentations
- Meet & greet/discussions with all Work Package leaders, Theme leaders and keynote speakers
- General poster session

**Presentation (EU) projects**

- *Tahar Ait-Ali:* Introduction to EuroPRRSnet
- *Cyril Gay:* Global Foot-and-Mouth Disease Research Alliance (GFRA): Future Goals and Direction

**13.00 – 14.30** **Lunch**

**14.30 – 15.00** **KEYNOTE**

|Hall B1|

*Dr Marvin J Grubman, Plum Island Animal Disease Center*

'The Role of the Leader Protein of Foot-and-Mouth Disease Virus as a Virulence Factor'

**15.00 – 17.00** **ORAL PRESENTATIONS**

| | **Emerging and Transboundary Diseases**<br>\|Hall B1\|<br>**Moderators:** *Dr Martin Beer, Fuat Özyörük* | **Theme 5: Intervention Strategies**<br>\|Hall B3\|<br>**Moderators:** *Dr Marie-Frédérique Le Potier, Prof Markus Czub* |
|---|---|---|
| 15.00 – 15.15 | *Uttenthal, Åse*<br>Novel trends to combat the transboundary animal diseases worldwide. Achievements and dissemination of the LAB-ON-SITE project of the EC | *Kumar, Mahesh*<br>Demonstration of Efficacy of Duvaxyn WNV under Field Conditions |
| 15.15 – 15.30 | *De Deken, Gill*<br>Micro-arrays as a molecular identification tool for possible BTV-8 vectors | *Rasmussen, Thomas Bruun*<br>Genetic stability of pestivirus genomes cloned into BACs |
| 15.30 – 15.45 | *Galleau, Stephanie*<br>Can vaccination prevent transplacental transmission of BTV-8 ? | *Vrancken, Robert*<br>Treatment of pigs with the antipestivirus compound BPIP reduces transmission of CSFV to untreated pigs |
| 15.45 – 16.00 | *Thuer, Barbara*<br>Toggenburg orbivirus (TOV): Genomic features, experimental infection, pathology and epidemiology of a novel bluetongue virus detected in goats from Switzerland | *Rodríguez, Fernando*<br>Immunization with a recombinant Baculovirus encoding a fusion of three African Swine Fever Virus (ASFV) antigens under the control of the mammalian CMV promoter protect pigs against a sublethal challenge with ASFV |

**16.00 – 16.30** **Coffee break**

| | | |
|---|---|---|
| 16.30 – 16.45 | *Froehlich, Andreas*<br>Bluetongue disease – Evaluation of the Monitoring and Surveillance System of the European Union | *Ortego, Javier*<br>Establisment of a bluetongue virus infection model in mice to evaluate vaccination strategies |
| 16.45 – 17.00 | *Van Rijn, Piet*<br>Bluetongue virus serotype 6 in the Netherlands | *Le Potier, Marie-Frédérique*<br>Improving the potency of mucosal immunisation using Ligand grafted PLGA Microspheres |

**17.00 – 18.30** **POSTER SESSION / MEET AND GREET AT MARKET / FAIR**

|Hall B2|

- Poster presentations
- Meet & greet/discussions with all Work Package leaders, Theme leaders and keynote speakers
- General poster session

**Presentation (EU) projects**

- *Gaëlle Kuntz-Simon:* Introduction to ESNIP2
- *Keith Sumption :* EUFMD, netspertise and FMD control: maintaining regional expertise needs a global network solution?

**17.30 – 18.30** **CLOSED SESSION**[*]

FLUTRAIN

|Room B10|

**20.00 - Late** **Beach Party**

|Beach|

[*] *This meeting is only accessible by invitation*

# Thursday, May 14th 2009

| | | |
|---|---|---|
| 8.00 – 9.00 | Registration | \|Lobby Hotel\| |
| | Poster drop off | \|Lobby Hotel\| |
| | Presentation drop off | \|Lobby Hotel\| |

**9.00 – 9.30** **KEYNOTE**

\|Hall B1\|

*Professor Aykut Ozkul, Ankara University*

'Life threatening Crimean Congo Haemorrhagic Fever in Turkey: Characteristics, Epidemiology and Future Perspectives'

**9.30 – 11.30** **ORAL PRESENTATIONS**

| | **Epizootic Diseases of Pig and Poultry**<br>\|Hall B1\|<br>**Moderators:** *Dr Linda Dixon, Dr William Dundon* | **Theme 6: Epidemiology and Surveillance**<br>\|Hall B3\|<br>**Moderators:** *Dr Claes Enøe, Dr Laura Powell* |
|---|---|---|
| 9.30 – 9.45 | *Kalthoff, Donata*<br>In vitro-induced HPAIV H5N1 escape mutants: In vitro and in vivo characterization | *Eblé, Phaedra*<br>Quantifying Foot-and-Mouth Disease and Swine Vesicular Disease Virus transmission parameters; collaborative output of EPIZONE WP 6.3 |
| 9.45 – 10.00 | *Brunhart, Iris*<br>Investigation of the epidemiology of avian influenza in wild birds at Lake Constance – a tri-national project of Germany, Switzerland and Austria | *Weesendorp, Eefke*<br>Airborne excretion and transmission of Classical swine fever virus |
| 10.00 – 10.15 | *Gale, Paul*<br>Novel Exposure pathways for poultry to H5N1 HPAI Virus from wild birds and poultry products | *Matras, Marek*<br>Monitoring of the fish viral diseases in Poland in 2004-2008- factors predisposing to dissemination of infectious agents |

**10.15 – 10.45** **Coffee break**

| | | |
|---|---|---|
| 10.45 – 11.00 | *Greiser-Wilke, Irene*<br>Classical swine fever – Eradication in the Americas, and cooperation with the OIE and European Union Reference Laboratory (OIE-EURL) | *Hagenaars, Thomas*<br>Risk Maps for the Spread of Highly Pathogenic Avian Influenza in Poultry |
| 11.00 – 11.15 | *Schroeder, Sabine*<br>Evaluation of CSFV Antibody ELISAs for the differentiation of infected from vaccinated animals | *Mathey, Alexander*<br>New Flubird Database - Platform for Data Exchange and Knowledge Building in Avian Influenza Surveillance |
| 11.15 – 11.30 | *Fernandez-Pinero, Jovita*<br>New molecular approaches for detection of African Swine Fever Virus | *Elbers, Armin*<br>To report or not to report: a psychosocial investigation aimed at improving early detection of Avian Influenza outbreaks |

**11.30 – 12.30 POSTER SESSION / MEET AND GREET AT MARKET / FAIR**

|Hall B2|

- Poster presentations
- Meet & greet/discussions with all Work Package leaders, Theme leaders and keynote speakers
- General poster session

**Presentation (EU) projects**

- *Michèle Bouloy:* Introduction to ARBO-ZOONET
- *William Dundon:* FLUAID – "Project achievements"

**12.30 – 14.00 Lunch**

**14.00 – 14.45 KEYNOTE**

|Hall B1|

*Dr Geneviève Libeau, Centre de coopération Internationale en Recherche Agronomique pour le Développement and*
*Professor Tom Barret, Institute for Animal Health, Pirbright Laboratory*
'Peste des petits ruminants virus (PPRV): Application of molecular technologies to understanding and controlling the disease'

## 14.45 – 16.45 CLOSED SESSION*

EuroPRRSnet
|Room A5|

## 14.45 – 16.45 ORAL PRESENTATIONS

| | **Epizootic Diseases of Ruminants**<br>\|Hall B1\|<br>**Moderators:** *Prof Volker Moennig, Dr Philippe Vannier* | **Theme 7: Risk assessment**<br>\|Hall B3\|<br>**Moderators:** *Prof Franz Conraths, Prof Alex Donaldson* |
|---|---|---|
| 14.45 – 15.00 | *Batten, Carrie*<br>Transplacental transmission of bluetongue serotype 8 (BTV-8) in the United Kingdom (2007-2008) | *De Vos, Clazien*<br>Quality of veterinary import risk analysis studies |
| 15.00 – 15.15 | *Rikula, Ulla*<br>Bluetongue surveillance in Finland 2007-2009 | *Mintiens, Koen*<br>A decision analysis model for the control of foot and mouth disease |
| 15.15 – 15.30 | *Van Rijn, Piet*<br>Bluetongue in the Netherlands: Preparedness on the unexpected | *Suszynski, Marcin*<br>Simulated surveillance sampling for foot-and-mouth disease |
| **15.30 – 16.00** | **Coffee break** | |
| 16.00 – 16.15 | *Ranz, Ana*<br>Approaches to DIVA assays for Bluetongue Diagnostic | *Wilking, Hendrik*<br>Highly mobile pathogens and their early detection-Possibilities and constraints of avian influenza surveillance in wild birds |

* *This round table discussion is only accessible by invitation*

| | | |
|---|---|---|
| 16.15 – 16.30 | *Bu, Zhigao*<br>Efficacy evaluation of goatpox virus vectored peste-des-petits-ruminants vaccine in Field | *Martinez, Marta*<br>Wild bird movements as a way of introduction of transboundary emerging diseases: the case of Crimean Congo hamorrhagic fever |
| 16.30 – 16.45 | *Parida, Satya*<br>Detection of FMD Infection in Vaccinated Animals (DIVA) in Turkey: Comparison of tests to detect FMDV carrier animals | *De Vos, Clazien*<br>Veterinary risk of deer in robust natural corridors in the Netherlands |

## 16.45 – 18.15 POSTER SESSION / MEET AND GREET AT MARKET / FAIR

|Hall B2|

- Poster presentations
- Meet & greet/discussions with all Work Package leaders, Theme leaders and keynote speakers
- General poster session

**Presentation (EU) projects**

- *Declan O'Brien:* Introduction to DISCONTOOLS and ETPGAH
- *Mari Smits:* EADGENE overview and results

## 16.45 – 17.30 CLOSED SESSIONS*

Discussion workgroup WP5.1 RVFV vaccines
|Room B10|

Discussion workgroup WP5.1 DIVA vaccines
|Room B11|

## 20.00 - Late Cocktail Pro Lounge

|Hall B2|

* *This meeting is only accessible by invitation*

# Friday, May 15th 2009

## OPEN SESSIONS

| | |Hall A1| | |Hall A2| |
|---|---|---|
| 8.45 – 13.15 | ARBO-ZOONET: WNV and CCHFV<br>*and*<br>IC6.6: BTV Epidemiology | *Sumption, Keith*<br>PCP to FMD freedom - concept and vision for stimulating risk based FMD surveillance and control at national, regional and global scale<br><br>GFRA workshop: FMD |

## CLOSED SESSIONS*

| | |Room A4| | |Room A6| | |Room A7| | |Room A8| | |Room A9| | |Room A10| |
|---|---|---|---|---|---|---|
| 9.00 – 13.00 | | | | | CF meeting |
| 9.00 – 10.00 | Discussion workgroup<br>WP5.1 **ASFV** vaccines | | Discussion workgroup<br>WP5.1 **BTV** vaccines | | | |
| 10.50 – 11.30 | WP5.4 | | WP5.1 | WP5.2 | WP5.3 | |
| 11.30 – 12.30 | | General Theme 5 discussion | | | | |
| 14.00 - 17.30 | | | IC 6.6 | | | |

* *These meetings are only accessible by invitation*

EPIZONE

# Keynotes

# KEYNOTE: PREDICTIONS, PROJECTIONS, AND PERSPECTIVES OF CLIMATE CHANGE

STOCK, MANFRED[1]

POTSDAM INSTITUTE FOR CLIMATE IMPACT RESEARCH[1]

Key words: climate change, scenarios, impacts, uncertainties, vulnerability assessment

Climate change has already speeded up showing more and more consequences with a vengeance. What do we know about the probable future progress and what kind of impacts shall we have to face? First of all there is no equivalent to weather forecasts. Present climate change - in contrast to those in Earth's history - highly depends on human decisions, and so do its consequences. Therefore we prefer to speak of projections or scenarios instead of predictions. Those decisions have to be made under severe uncertainties and the only certainty is that waiting for certainty is no option. Which are the options to decide? A valuable option is to assess the vulnerability of a climate-sensitive system consisting e.g. of pathogens, host organisms, environment and civilization with respect to possibly critical climate stimuli. The advantage of vulnerability assessments is the identification of adverse or beneficial measures showing perspectives of management options. One could say: climate change is not a matter of predictions but of management.

# KEYNOTE: THE ROLE OF THE LEADER PROTEIN OF FOOT-AND-MOUTH DISEASE VIRUS AS A VIRULENCE FACTOR

GRUBMAN, MARVIN J.[1]; DIAZ-SAN SEGUNDO, FAYNA[1]; ZHU, JAMES[1]; KOSTER, MARLA[1]; DIAS, CAMILA C. A.[1]; DE LOS SANTOS, TERESA[1]

USDA, ARS, PLUM ISLAND ANIMAL DISEASE CENTER[1]

Key words: FMDV, leader protein, virulence

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals that is spread by contact or aerosol. The viral genome is a positive-sense single-stranded RNA molecule and is translated into a polyprotein which is processed by the viral encoded proteinases, L, 2A, and 3C, into the mature structural and nonstructural proteins. Translation initiates internally in a cap-independent manner directed by the internal ribosome entry site. The first translation product, Lpro, is a papain-like proteinase that cleaves itself from the growing polypeptide chain and also cleaves the translation initiation factor eIF-4G, which is required for translation of capped mRNAs. Because translation of FMDV mRNA is cap-independent, cleavage of eIF-4G by L results in the shut-off of only host cell protein synthesis.

Since Lpro has a critical role in inhibition of host protein synthesis, we hypothesized that a virus lacking the coding region for this protein would be less virulent. We constructed a genetic variant of FMDV lacking the complete L coding region. The leaderless virus, A12-LLV2, replicates slightly more slowly than wild-type (WT) virus in BHK-21 cells. However, in contrast to WT virus, A12-LLV2 is highly attenuated in both bovines and swine. After aerosol exposure of cattle to A12-LLV2 it did not spread systemically beyond the initial site of infection in the lungs. In contrast, WT virus caused extensive local infection and rapidly spread to secondary sites.

We found that supernatants of leaderless virus infected primary cells contained higher levels of antiviral activity than supernatants from WT virus infected cells and this activity was type I interferon (IFNalpha/beta) specific. These results suggested that Lpro blocks the innate immune response by inhibition of translation of IFNalpha/beta mRNAs. Further studies demonstrated that leaderless virus infection also results in significantly higher levels of IFNbeta mRNA synthesis than in WT virus infected cells and these higher levels correlate with enhanced induction of various IFN-induced genes. Nuclear factor kappa B (NF-kappaB), a latent transcription factor required for induction of IFNbeta mRNA, is degraded in WT FMDV infected cells, but not in leaderless virus infected cells. Cleavage of NF-kappaB correlates with the nuclear translocation of Lpro, occurs in the absence of other FMDV proteins, and requires L proteinase activity. Bioinformatic analysis of Lpro suggests that it contains a domain associated in some cases with nuclear retention of molecules involved in transcriptional control (SAP domain). Mutation of two conserved residues in this domain resulted in a stable mutant virus that displayed an attenuated phenotype. While Lpro initially translocated to the nucleus, it was not retained in this compartment and mutant virus infection resulted in the degradation of eIF-4G but not NF-kappaB. Preliminary data indicates that the SAP mutant virus is avirulent in swine and does not result in viremia, but does induce an FMDV-specific neutralizing antibody response. Thus Lpro functions as a virulence factor by disrupting the host cell at both the transcriptional and translational levels.

# KEYNOTE: LIFE THREATENING CRIMEAN CONGO HAEMORRHAGIC FEVER IN TURKEY: CHARACTERISTICS, EPIDEMIOLOGY AND FUTURE PERSPECTIVES

OZKUL, AYKUT [1]

ANKARA UNIVERSITY, FACULTY OF VETERINARY MEDICINE[1]

Key words: Crimean-Congo Haemorrhagic Fever Virus, Turkey, Epidemiology

Crimean–Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by an arbovirus Crimean–Congo hemorrhagic fever virus (CCHFV), which is a member of the Nairovirus genus (family Bunyaviridae). CCHF was first recognized during a large outbreak among agricultural workers in the mid-1940s in the Crimean peninsula.

CCHF has been causing clinically severe disease in human being in Middle Anatolia and Black Sea Region of Turkey since 2002. CCHF cases are more prevalent during late spring and summer. In Turkey, more than 2500 clinical cases with 5-50% fatality rate have been reported between 2002 and 2008 by Ministry of Health. Turkey seems to be one of the countries in which the world's biggest outbreak of CCHF has occurred. The analysis of the latest epidemics (in 2008) showed that the disease has almost spilled-over the country by infecting wider areas in contrast to those in previous years. Clinical symptoms recorded in local cases are characterized by fever, head ache, myalgia, weakness, fatigue and lack of appetite, which are followed by nasal bleeding and/or ecchymotic hemorrhages in various parts of skin. In addition nausea, vomiting, abdominal pain and diarrhea can also be seen in patients.

Although the virus has been isolated from blood samples of CCHF patients, currently, there is little information on genetic diversity and in relation molecular epidemiology of the currently circulating CCHF virus/viruses. Based on limited evidences obtained from S and M segment sequences, the local virus was found similar to those reported in southwestern Russia and Kosovo. The most recent analysis based on sequence data from last three year's viruses revealed there are two different clades were detected in Turkey.

Hyalomma marginatum, main vector of CCHFV, was found predominantly (49.9%) on ruminant species in epidemic areas in Turkey. In addition, seroprevalance of CCHFV was detected as 79% in cattle serums collected from endemic regions.

Diagnosis of the infection is performed widely in regional governmental and/or university hospital laboratories using various ELISA systems and in vitro amplification techniques including real time and in house RT-PCR.

In a future perspective, several research groups have already focused to produce subunit vaccine(s) for use for prophylactic purposes in endemic and/or risk groups and/or areas. On the other hand, couple of groups is also working on production of hyperimmune serum using purified Gn and Gc glycoproteins encoded by M segment in order not only to help accelerating clinical recovery of CCHF patients but also to calm people down after possible tick bites during coming CCHF sessions.

# KEYNOTE: PESTE DES PETITS RUMINANTS VIRUS (PPRV): APPLICATION OF MOLECULAR TECHNOLOGY TO UNDERSTANDING THE BIOLOGY AND CONTROL OF THE DISEASE

BARRETT, THOMAS[1]

INSTITUTE FOR ANIMAL HEALTH (IAH)[1]

Key words: PPR, Vaccine, Reverse Genetics, Diagnostics

Peste des petits ruminants (PPR) is highly contagious disease of small ruminants first described in West Africa in the 1940s. Infection of sheep and goats can result in mortalities approaching 90%. As a consequence its introduction into naïve herds can be devastating with profound effects on the rural economies of the countries concerned. The infectious agent, a virus in the morbillivirus genus of the family Paramyxoviridae, is closely related to rinderpest virus (RPV) which causes a similar disease in cattle and other large ruminants. Whilst rinderpest has almost certainly been eradicated globally as a result of a concerted vaccination campaign carried out over the past quarter of a century, PPRV has spread from what was thought to have been its original home in sub-Saharan Africa to The Middle East and southern Asia and from there on to China and many neighbouring countries of the former Soviet Union. Most recently it has caused many deaths in sheep and goats in Morocco. The control and possible elimination of PPR is one of the main targets of the United Nation Food and Agriculture Organisation's (FAO) Emergency Preventive System (EMPRES) programme which fosters "the effective prevention and progressive control of plant and animal pests and diseases, including especially those which are of transboundary nature". PPR is considered as one of the main animal transboundary diseases which constitute threats to livestock production in many developing countries. Although an effective vaccine and reliable diagnostic tests are available, improvements such as the development of DIVA vaccines and real time RT-PCR assays, will greatly aid future control programmes. In this presentation the application of molecular technologies to achieve a better understanding of the virus and its control will be addressed.

The most valuable molecular technology used to study the biology of negative strand viruses is reverse genetics. A full reverse genetics system has been developed for many negative strand viruses, including RPV, and this has been applied to the development of DIVA vaccines for both RPV and PPRV, however, to date only a minigenome rescue system is available for PPRV. We have used this "minigenome" rescue system to study the ability of proteins from other morbilliviruses to interact with those of PPRV. In addition, sequencing of the complete genomes of the vaccine and some virulent strains of the virus has highlighted some mutations in the promoter region of the virus which may, by analogy to RPV, be important determinants of virulence. Two real time RT-PCR assays have been developed for the specific detection of PPRV which are less prone to problems of cross contamination and which are more sensitive than conventional RT-PCR. These will enable a much more rapid diagnosis of the virus and can be easily scaled up to analyse large numbers of clinical samples.

# KEYNOTE: FIRST OCCURRENCE OF PESTE DES PETITS RUMINANTS DISEASE IN MOROCCO

LIBEAU, GENEVIÈVE[1]; KWIATEK, OLIVIER[1]; MINET, CÉCILE[1]; EL HARRAK, MEHDI[2]; DIALLO, ADAMA[3]; ALBINA, EMMANUEL[1]

CENTRE DE COOPÉRATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT (CIRAD)[1]; LABORATOIRE BIOPHARMA -DIRECTION DE L'ELEVAGE[2]; FOOD AND AGRICULTURE ORGANIZATION[3]

Key words: PPRV, emergence, Morocco, PCR, lineage IV

Peste des petits ruminants virus (PPRV) has a geographic range extending from western Africa to eastern and southern Asia but until recently was unknown in North Africa. Using the latest diagnostic methodologies, the first occurrence of PPR disease in Morocco was confirmed by CIRAD, an OIE/FAO reference laboratory for PPR. PPR in Morocco poses a risk for the rest of Maghreb as well as for southern European countries that maintain close ties with this region. Positive results for PPRV infection were obtained from blood and tissue samples received soon after the first reports of the outbreak, by conventional PCR (Couacy-Hymann et al. 2002) and by a newly developed real-time PCR based on the TaqMan method (unpublished work). Both these tests target the N gene, the most abundantly transcribed of the virus genes. Molecular characterization of PPRV, based either on sequences derived from the N gene (Work at CIRAD) or the F gene (Shaila et al, 1996), shows the existence of four lineages of the virus, lineages I to IV. Partial sequencing of the amplicons from infected tissue samples from the Moroccan epizootic confirmed that the causative agent was PPRV of lineage IV, a lineage circulating in The Middle East and Asia but not reported before from Africa. The Moroccan virus is very closely related to viruses found earlier in Saudi Arabia and in Iran and differs significantly from the 3 other lineages previously found in Africa. Over the past 5-8 years, there has been a steady expansion of the disease in Africa, apparently toward east and south. To better understand the factors driving the emergence of lineage IV in Morocco, we analysed infected materials sent in 2008 from other countries in Africa for laboratory confirmation after clinical suspicions of PPR disease were reported in goats. We compared these samples to previously characterized African viruses (Kwiatek et al, 2007). The Sudan 72 isolate, one of the historical strains, as well as the most recent isolate obtained from Tanzania, were placed in eastern African lineage III, whereas a more recent isolate from Sudan was identified as belonging to Asian lineage IV. From the molecular comparison of strains, we have shown that the stepwise progression of the disease into Tanzania, the first occurrence in that country, involved lineage III virus but that this genotype has now been replaced in Sudan by Asian lineage IV virus. PPRV needs close contact for transmission between infected and naïve animals, therefore this new information clearly indicates that the virus was introduced into North Africa through movement of live infected animals. Many of the people in this region of Africa lead a nomadic lifestyle and this greatly favours the spread of viruses and other diseases across the continent. The emergence of PPR in Morocco, and its extension in Africa, is certainly linked to the expansion of trade in animals and subsequent movements of these animals into previously disease free regions.

EPIZONE

# Projects

# EUROPRRSNET, A PLURIDISCIPLINARY NETWORK FOR UNDERSTANDING AND COMBATING PRRS IN EUROPE

AIT-ALI, TAHAR[1]

UNIVERSITY OF EDINBURGH[1]

Key words: PRRS, pig, network, Europe

The emergence of porcine reproductive and respiratory syndrome (PRRS), a potent viral disease of pigs, has had a major impact on the health and welfare of pigs throughout the world. Its appearance in Europe in the early 1990's, has resulted in a significant increase in the number of early farrowing, late-term abortions, along with increased mortalities in neonates in breeding herds and respiratory disease in nursery and fattening units. Changes in husbandry have lessened the impact of PRRS in herds but incidents of severe disease attributed to this virus regularly occur. More than 20 years since its first emergence, PRRS disease is still having a major impact on pig health and welfare. PRRS accounts for about a third of the cost of infectious disease to the US pig industry, ca. $600 million annually. The detrimental economic impact of PRRS in Europe is currently not quantified, though is also expected to be very substantial. Due to a lack of effective control measures in Europe the disease is now endemic in many countries.

The recent discovery that an outbreak of "high fever pig disease" in China was caused by a highly pathogenic, variant strain of the American genotype of the PRRS virus (PRRSV), has caused much consternation. Outbreaks in 2007 in 10 eastern provinces of China are reported to have killed 400,000 of the 2,120,000 infected pigs in 4 months. This form of the disease is also reported to have spread to other countries, including Vietnam, Russia and Bhutan. Similarly, Sweden, reputed to be a PRRS-free country, faced its first outbreaks of PRRS in 2007. These are clear reminders that the control of PRRS is important for the industry as well as the food chain and ultimately to the consumer.

PRRS is a large, scientifically-challenging current problem for Europe, which requires a well co-ordinated solution. PRRS research is actively funded at national levels however Pan-European capacity-building activities do not currently exist to promote active scientific exchange of strategies. EuroPRRSnet offers an appropriate stepping stone networking mechanism for the European PRRS research community. The launch of a EuroPRRSnet is highly desirable and relevant to the current need of the PRRS community as it will integrate and develop timely strategies among European participants to combat the disease. The network currently involves 12 countries and 20 institutions. The aim of this initiative is to develop more effective multidisciplinary collaborative PRRS research centred on PRRSV epidemiology, immunopathology, vaccine development and harmonization of diagnostics tools. With a specific emphasise on genetics and genomics this network will improve understanding of, and hence better control, of PRRS. The intention of the initiative is to generate productive and concrete outcomes to maximise the breadth of the European collaboration. EuroPRRSnet will establish a database of biological and bioinformatics resources relevant to PRRS research to enable effective sharing between national and ultimately international programmes. The strategies derived from this Action will benefit animal health, producers, public health and allied organizations that have a stake in animal agriculture systems. The recommendations will be widely disseminated and serve as a roadmap for training and future initiatives.

# GLOBAL FOOT-AND-MOUTH DISEASE RESEARCH ALLIANCE (GFRA): FUTURE GOALS AND DIRECTION

GAY, CYRIL[1]; RODRIGUEZ, LUIS[1]; JEGGO, MARTYN[2]; ALEXANDERSEN, SOREN[3]; CHARLESTON, BRYAN[4]; PATON, DAVID[4]; KROGH NIELSEN, THOMAS[5]; BELSHAM, GRAHAM[5]; DEKKER, ALDO[6]

AGRICULTURAL RESEARCH SERVICE[1]; AUSTRALIAN ANIMAL HEALTH LABORATORY [2]; NATIONAL CENTRE FOR FOREIGN ANIMAL DISEASE[3]; INSTITUTE FOR ANIMAL HEALTH (IAH)[4]; NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[5]; CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[6]

Key words: FMD, Research, Alliance, Objectives, Vaccines, Diagnostics

Introduction: A group of international animal health scientists (1) met on Plum Island May 2008 to define the purpose and goals of the Global Foot-and-Mouth Disease Research Alliance (GFRA). The group agreed that the purpose of the GFRA should be to establish a coordinated global alliance of scientists to produce evidence and innovation that will enable the progressive control and eradication of Foot-and-Mouth Disease (FMD). The group also agreed that the following five strategic goals should drive the work of the GFRA: 1) Facilitate research collaborations ; 2) Conduct strategic research to better understand FMD; 3) Development of the next generation of control measures and strategies for their application; 4) Determine social and economic impacts of new generation of improved FMD control; and 5) Provide evidence to inform development of policies for safe trade of animals and animal products in FMD endemic areas.

Discussion: There are currently no research laboratories with the necessary critical mass and support structures to achieve the GFRA strategic goals. It is therefore imperative that laboratories worldwide with active FMD research programs work together to reach the critical mass needed to achieve the GFRA goals. Critical will be to establish research programs that will meet the needs of countries that are endemic for FMD and that are the most affected by the devastating economic impact of this disease. The current members of the GFRA have therefore agreed to the following action plan: 1) identify partnership opportunities and promote funding of collaborative research projects; 2) expand and coordinate the alliance; 3) promote mechanisms and bring together the necessary experts to do gap analysis and set research priorities; 4) organize and manage GFRA and related meetings including issues of sponsorship; and 5) seek funding for GFRA coordination activities.

Next Steps: The GFRA will hold a workshop on the last day of the EPIZONE meeting in Turkey on May 15, 2009, recruit new members, review current FMD research projects, solicit input on research priorities, and establish new strategic research collaborations to advance the progressive control and eradication of FMD.

(1)C. Gay, L. Rodriguez, R. Moore, USDA/ARS-USA; J. Hammond, M. Jeggo, CSIRO-Australia; B. Charleston, D. Paton- Pirbright-UK; B. Perry, Nuffield Department of Clinical Medicine, University of Oxford, UK; P. Kitching-CFIA-Canada; A. Dekker- Central Veterinary Institute of Wageningen-Netherlands; K. DeClerq, CODA-CERVA-VAR-Belgium; S. Alexandersen, National Veterinary Institute- Denmark; R. Drummond, Food & Rural Affairs-UK

# EADGENE OVERVIEW AND RESULTS

AYUSO, SANDRINE[1]; CHANNING, CAROLINE[2]; PINARD VAN DER LAAN, MARIE-HÉLÈNE[1]; SMITS, MARI[3]

L'INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE (INRA)[1]; UNIVERSITY OF EDINBURGH, ROSLIN INSTITUTE[2]; ANIMAL SCIENCES GROUP[3]

Key words: host-pathogen interactions, Animal Health , Genomics, mastitis, salmonella

European Animal Disease Genomics Network of Excellence for Animal Health and Food Safety
EADGENE is a Network of Excellence (1 September 2004 to 28 February 2010), funded by the EC, which coordinates a genomics approach to the unraveling of host-pathogen interactions, thereby providing the basic knowledge necessary for the development of new or improved therapeutics and vaccines, improved diagnostics and the breeding of farm animals for disease resistance. Our innovative research projects are designed to complement existing projects between 15 leaders in this field. This research will impact upon human health and lifestyle choices by including on pathogens of importance in the food chain.

Integrating activities are sustainable for sharing skills.
Integrating tools such as technological resources, bioinformatics and analytical tools have been created. EADGENE has established a database of biological resources and provided oligo microarrays for chicken, cattle and pigs, which are available to all partners in the Network of Excellence (NoE) and are used as our common preferential tools. Our laboratories have adopted common QC standards and methods of analysis in genomics and bioinformatics. More than 30 researchers have benefited from funding for visits to a partner laboratory to learn about and share cutting edge genomics techniques.

Four research areas :
Research activities are structured into four multidisciplinary themes (1) structural genomics (genes, miRNA, regulating mechanisms of resistance), (2) population genetics (models and relevant phenotypes, QTL), (3) functional genomics on mastitis (cattle, goats, sheep), salmonellosis (pig, poultry), infections caused by Escherichia coli EHEC (cattle) and viral models (IPNV and ISAV in salmon and trout), (4) operational genomics (meta-analysis). These collaborative studies compare and use the wealth of models (different hosts, pathogens, infections ...) using common genomic tools

Technology transfer and Dissemination to general public :
A special effort is dedicated to promoting technology transfer which bridges the gap between research and industry. Three concrete activities are performed: the ontology of important traits; the development of databases from private companies; and two projects on poultry resistance. Websites and newsletters are dedicated to industry, the scientific community and the general public.
The ethical implications of animal health genomics are a fundamental component of EADGENE. Workshops and courses are regularly organized. The next yearly "EADGENE days" brings together animal health genomics research scientists and industries, and will be held on 13-15 October 2009, Paris.

Benefits for EADGENE researchers :
EADGENE partners have benefited from the increased access to resources, knowledge, staff recruitment, and scientific cooperation. The network has been a catalyst and unifier for the development of other national or European contracts such as SABRE, FABRE, NADIR...

# INTRODUCTION TO ETPGAH & DISCONTOOLS

O' BRIEN, DECLAN[1]

ETPGAH[1]

Key words: disease control stakeholders prioritise animals

The European Technology for Global Animal Health (ETPGAH) was established in December, 2004 with the objective of identifying the most critical issues that need to be addressed in order to control diseases in animals. The ETPGAH was funded by the European Commission and led by industry. All stakeholders were invited to participate and developed a Vision, Strategic Research Agenda (SRA) and an Action Plan.

The Vision foresees the speedier development of tools for disease control by focusing our research effort on the most important gaps in the most important diseases. These may be emerging, established or zoonotic diseases. By delivering better disease control, animal health and welfare is protected, human health benefits in terms of zoonotic disease control but human health also benefits from food security, safety and quality.

In developing the SRA, the stakeholders identified 6 major themes:
1. Prioritisation of Animal Diseases
2. Gap Analysis
3. Fundamental Research
4. Enabling Factors
5. Regulatory Issues
6. Global Perspective

It was recognised that we need to prioritise our effort and focus on finding new disease control tools by collaborative research on a limited number of critical targets. Critical to this concept is the need to identify and prioritise the most important gaps in our ability to control the critical diseases.

It is vital that we have the fundamental research capacity – infrastructure and people – to carry out the necessary research. Along with filling gaps, efficiency can also be improved by avoiding unnecessary capacity development. Enabling factors such as quality assurance, intellectual property rights and facilitation of technology transfer are critical components in moving from basic research to the development of a tool that can be used to fight a disease.

The correct regulatory environment is vital to stimulate innovation. A balance needs to be reached between protecting human and animal health from the risks associated with a product versus the wish to eliminate all hazard.
From a global perspective, it is in the interests of everybody to reduce the global burden of disease.

Having explored the broad areas that need attention in the SRA, the Action Plan was then developed and published in July, 2007 identifying the actions – research or information gathering – that need to be carried out in order to deliver the SRA. The purpose of the ETPGAH is to now oversee the delivery of the Action Plan.

DISCONTOOLS, as a project, responds to the critical recommendation in the Action Plan to provide a mechanism to identify the most important diseases, identify the most important gaps in our ability to control these diseases and focus research on these gaps. In addition, DISCONTOOLS is exploring how to deploy new technologies in the animal health research area at the earliest opportunity.

DISCONTOOLS will work on approximately 45 diseases gathering data, carrying out gap analysis and developing a public web based priotitisation model based on criteria including disease knowledge, impact on wider society, impact on public health, impact on trade, animal welfare as well as effectiveness of control tools.

Being web based, the information will be dynamic providing the opportunity for interested parties to comment and for the prioritised diseases to be updated on an ongoing basis. DISCONTOOLS is scheduled to conclude its work by March, 2012.

# THE EU PROJECT "FLUAID"

DUNDON, WILLIAM[1]; CAPUA, ILARIA[1]

INSTITUTO ZOOPROFILATTICO SPERIMENTALE VENEZIE[1]

Key words: Avian influenza, EU project

The primary goal of the FLUAID project, which began in January 2006 with European funding of €1.2 million, has been the joint development and application of novel technologies to combat Avian influenza infections. These goals have been achieved through the interaction of leading European institutes along with the active collaboration of Asian laboratories. The first deliverables of the project have included the identification of prototype strains for vaccine production and the development and validation of three companion diagnostic tests which can be used in combination with the DIVA (Differentiating Infected from Vaccinated Animals) strategy. The validation of penside tests and Real-time PCR protocols has also been a part of the project. Studies have also generated data on the presence of virus in the meat of vaccinated versus unvaccinated birds. To complement this work, studies on pathogenesis in reservoir species (e.g. quail), species tropism and the genetic and antigenic variability of endemic strains has been performed. Quantitative transmission studies have been a key part of the project with experiments being performed in different species including Pekin and Muscovy ducks. To date, the data generated by the FLUAID project has been presented at international meetings and published in 9 peer-reviewed journals.

# THE EUROPEAN SURVEILLANCE NETWORK FOR INFLUENZA IN PIGS (ESNIP)

KUNTZ-SIMON, GAËLLE[1]; KYRIAKIS, CONSTANTINOS S.[2]; FONI, EMANUELA[3]; MALDONADO, JAIME[4]; LOEFFEN, WILLIE[5]; BROWN, IAN H.[6]; ESSEN, STEVE[6]; MADEC, FRANÇOIS[1]; MATROSOVICH, MIKHAIL[7]; BUBLOT, MICHEL[8]; CHENCHEV, IVAYLO[9]; PEIRIS, MALIK[10]; OLSEN, CHRIS[11]; VAN REETH, KRISTIEN[2]

AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[1]; GHENT UNIVERSITY[2]; INSTITUTO ZOOPROFILATTICO SPERIMENTALE[3]; LABORATORIOS HIPRA S.A[4]; CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[5]; VETERINARY LABORATORIES AGENCY (VLA)[6]; PHILIPPS UNIVERSITÄT MARBURG[7]; MERIAL S.A.S.[8]; NATIONAL DIAGNOSTIC RESEARCH VETERINARY INSTITUTE[9]; UNIVERSITY OF HONG-KONG[10]; UNIVERSITY OF WISCONSIN-MADISON[11]

Key words: pig, influenza virus, reassortant, molecular epidemiology, serosurveillance

Swine influenza is an important cause of acute respiratory disease in pigs and pigs are considered as an intermediate host for the transmission of influenza viruses to humans. While surveillance networks for human, equine and avian influenza have been established decades ago, surveillance for swine influenza has long been neglected. The "European Surveillance Network for Influenza in Pigs 2" (ESNIP 2) was a co-ordination action (SSPE-CT-2005-022749, January 2006 – March 2009) funded by the European Commission in the 6th Framework Research Programme. It maintained and expanded the surveillance network established during ESNIP 1 (2001-2004) and aimed to improve our knowledge of the epidemiology and evolution of swine influenza viruses (SIVs) in Europe. During the 3-year period of the project, virological and serological surveillance have been conducted in parallel in six European countries. The data confirmed that SIVs of H1N1, H3N2 and H1N2 subtypes are co-circulating among European pigs. Still, there were differences in the prevalence of each subtype on regional or national levels, with little if any H3N2 activity in the UK or Brittany (France). No major antigenic changes in the hemagglutinin proteins of each SIV subtype were detected. However, novel reassortant viruses between the first generation H1N2 reassortants and avian-like swine H1N1 viruses were occasionally detected in Italy and France. These data will be used to optimise the diagnosis and control of swine influenza. European swine influenza researchers also started to liaise with researchers in the US and Asia with the purpose to compare the epidemiology of swine influenza on different continents. Furthermore, the ESNIP consortium has been working on improved methods for the serological detection of avian influenza in pigs. These initiatives and interactions are consistent with improved pandemic preparedness and planning for human influenza.

EPIZONE

# Oral presentations

# ORAL: NEW STRATEGIES IN KOI HERPESVIRUS (KHV) DIAGNOSTICS RECOGNIZING LATENT INFECTED CARRIER FISH

BERGMANN, SVEN M.[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: KHV, latency, semi-nested PCR

The infection with the koi herpesvirus (KHV, syn. CyHV-3) represents an emerging disease agent for carp and koi (Cyprinus carpio) aquaculture world-wide. In Germany, outbreaks of KHV disease (KHVD) and detections of the causative viral agent increased from 2002 to 2008 in eatable carps as well as in imported ornamental kois. Especially in extensive carp aquaculture, mortality rates of up to 80% occurred in harvested fish in 2008. This led to financial losses of between 200.00 and 900.000 €, depending on farm size and production cycle.
On problem is the behaviour of KHV which induces latency or persistence in infected fish. In this phase of viral manifestation, very weak virus loads (5 – 10 particles) are found in kidney, leukocytes and gill tissues. Most of the diagnostic tools are not able to identify KHV in this weak concentration. These unrecognized infected animals represent a real threat for naïve, not infected carps or kois of each size and age.
To overcome the gaps in KHV diagnostics in terms of identification of "apparently healthy" latent / persistent infected carriers, a workshop was held in Germany in 2008. Specialists form fish health services and colleagues from regional diagnostic laboratories of nearly all German federal states summarized the results as followed:
1. at least 10 fish larger than 5 cm shall be samples in two pools (from smaller fish two pools with 10 fish) (OIE recommend now not to pool more than two fish in one sample)
2. gills and kidney are the most prominent KHV target organs for lethal sampling
3. gill swabs and separated leukocytes can be used for non-lethal sampling
4. fish shall be caught and separated for at least 24 hours but not longer than seven days before sampling (alternative a real or a simulated transportation (netting) can be used)
5. samples shall be investigated by realtime PCR (Gilad et al. 2004) or nested PCR (Gilad et al. 2002 followed by Bergmann et al. 2006)
6. if possible fish shall be investigated as a single pool (gills and kidney)
7. no pools shall be done with gill swabs and / or leukocytes (single fish testing)
The diagnostic sensitivity of realtime and nested PCR in single fish samples ranged in KHV DNA recognisation between 5 and 10 genomic equivalents. Due to contamination risk, nested PCR results considered only to be positive when all controls (preparation controls from negative fish and all water controls) are negative. This is also valid for realtime PCR.
To minimize the possibilities of contamination in laboratory, a semi-nested PCR based on the major glykoprotein gene of KHV was developed, partly validated and compared with results of other PCRs for sensitivity and specificity.
The new semi-nested PCR reached an equal diagnostic sensitivity of 5 to 10 genomic equivalents and is specific to KHV only.
As heterologous test controls carp pox virus (CyHV-1), goldfish haematopoietic necrosis virus (CyHV-2), Channel catfish herpesvirus (CCV) and herpesvirus anguilla (HVA, Ang-HV 1) DNAs were utilized. No signal occurred in gels after semi-nested PCR when these DNAs were used.

# ORAL: SIMULATED SURVEILLANCE SAMPLING FOR FOOT-AND-MOUTH DISEASE

SUSZYNSKI, MARCIN[1]

UNIVERSITY OF CALIFORNIA, DAVIS[1]

Key words: FMD, foot-and-mouth disease, surveillance sampling

The objective of the study was to compare time-specific probabilities of capturing a herd infected with FMD virus for several surveillance sampling schemes that varied according to sample size, sampling frequency, and type of sampling allocation employed. For each simulated epidemic of FMD, a simple random sample of herds was obtained at each day of the epidemic using Monte Carlo sampling, and the probability, Pt, was estimated for each sampling scheme as the daily probability of capturing at least one infected herd in 10,000 simulations. The probability, Ct, of a sampling scheme capturing at least one infected herd by day t, was the probability of capturing an infected herd by day t-d, where d was the number of days since the last sample collection, plus the probability that a sample taken at day t would capture an infected herd (Pt), given that an infected herd had not yet been captured by the time of the previous sample collection. Results of the stepwise multiple linear regression indicated that all three variables (sample size, type, and frequency of sampling) were significantly associated with the probability of capturing at least one infected herd at day 21. The main findings of the study were that the risk proportional sampling strategies that included the high risk sales yards provided the highest probabilities of capturing an infected herd. The risk proportional sampling without sales yards and simple random sampling approaches were less likely to capture an infected herd, and the probabilities of capture for the two methods did not differ much from each other, regardless of frequency of sampling, particularly before day 14 of the epidemic. Increased frequency of sampling and large sample size per se increased the probability of capturing an infected herd at a given time.

# ORAL: TRANSPLACENTAL TRANSMISSION OF BLUETONGUE SEROTYPE 8 (BTV-8) IN THE UNITED KINGDOM (2007-2008)

BATTEN, CARRIE[1]; DARPEL, KARIN [1]; VERONESI, EVA [1]; WILLIAMSON, SUSANNA[2]; ANDERSON, PETER[3]; DENNISON, MIKE[1]; CLIFFORD, STEWART[3]; SMITH, CIARAN [3]; PHILLIPS, LUCY[3]; BACHANEK-BANKOWSKA, KASIA[1]; SANDERS, ANNA[1]; BIN-TARIF, ABID[1]; WILSON, ANTHONY[1]; GUBBINS, SIMON[1]; MERTENS, PETER [1]; OURA, CHRIS[1]; MELLOR, PHILIP[1]

INSTITUTE FOR ANIMAL HEALTH (IAH)[1]; VETERINARY LABORATORIES AGENCY (VLA)[2]; ANIMAL HEALTH[3]

Key words: transplacental transmission, Bluetongue serotype 8

Introduction

In 2006/2007 farmers across northern Europe reported increased incidence of abortion, stillbirth and weak calves in herds where BTV-8 infection had occurred. While clinical disease, especially fever in pregnant dams can result in the above, it was feared that BTV-8 was able to cross the placental barrier and infect foetuses in utero. A study investigating evidence for BTV-8 transplacental infection was performed in the UK.

Material & Methods:

Cohort: Cattle of breeding age, previously infected with BTV-8 in 2007 (between the arrival of BTV-8 in August and the start of the vector free period), which tested positive for BTV antibodies were identified in the UK. Calves born to these BTV-infected dams during the vector free period (VFP) (15/12/07 -15/03/08) were blood sampled (EDTA and serum) in March 2008. At the end of March there was still no evidence of BTV-8 circulation in the UK, so calves born up to the end of May were sampled within three days of birth and included in the study. Wherever possible dams were sampled alongside their calves to assess their current status of BTV infection. Calves which tested positive for the presence of BTV RNA were followed up through repeated sampling at 2-3 week intervals.

RNA Extraction and Real-time PCR: Tissues (spleen, lymph nodes) were homogenised in cell culture media using mortar and pestle. Where possible, material from the placental cotelydon (foetal side of the placentome) was collected and processed as for other tissue samples. RNA was extracted from either EDTA blood or tissue samples using either the MagnaPure (Roche) or the Universal (Qiagen) extraction robot, 'total NA/External_lysis' or 'One for all' protocol respectively; based on the manufacturers instructions. Real-time RT-PCR (rRT-PCR) was performed using a modified version of the procedure described by Shaw et.al. 2007.

C-ELISA: The detection of BTV specific antibodies in serum was carried out using the Pourquier C-ELISA kit.

Results:

A total of 21 out of 61 calves were shown to be infected with BTV by detection of BTV RNA by rRT-PCR. This equates to a transplacental transmission rate of 33% during the course of this study. In 52 cases dams and calves were tested in pairs to identify the current status of the dam in relation to the calves. In all cases the viral RNA load in the calves exceeded that of the dam. Seven dams had already cleared BTV infection. All but 3 calves had antibodies to BTV as assessed by ELISA. Of the calves that tested positive by rRT-PCR five (out of 21) were health compromised, two calves were born weak and died, another was stillborn and two calves displayed "dummy calf syndromes". Viral RNA in all calves declined over time, with almost all calves testing rRT-PCR negative at the end of the study.

The probability of transplacental transmission followed a linear pattern with it being more likely to occur in dams infected later rather than earlier in gestation.

Discussions & Conclusions

BTV-8 is capable of crossing the placental barrier to infect the foetus. In the UK, in 2007 this occurred in 33% of cases where the dam was infected with BTV-8 during gestation. Statistical analysis showed that transplacental infection occurred significantly more often later in gestation. Evidence of lower transplacental infection rates have been described elsewhere in Europe and these data along with the data from the UK will be discussed.

# ORAL: AIRBORNE EXCRETION AND TRANSMISSION OF CLASSICAL SWINE FEVER VIRUS

WEESENDORP, EEFKE[1]; LOEFFEN, WILLIE[1]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]

Key words: Air sampling; Airborne transmission; Classical swine fever virus; Virus excretion

Objectives

During epidemics of Classical Swine Fever (CSF), the route of virus introduction into a farm is often unclear. Beside traditional routes of transmission, one of the suggested routes is via the air. Animal experiments suggest that this is a possible route of transmission, although virus detection from the air was, until recently, unsuccessful. To further investigate a possible role of airborne spread, we quantified the amount of virus emitted in the air by infected pigs. Furthermore, the airborne transmission over a short distance was studied.

Materials and Methods

Two animal trials were performed. The first trial consisted of four transmission experiments with 10 pigs each. In each experiment, 3 pigs were inoculated with either the low virulent strain Zoelen, a low or high dose of the moderately virulent strain Paderborn, or the highly virulent strain Brescia. The other 7 pigs in each experiment served as contact pigs. Each experiment was conducted in a separate isolation unit. At several moments after infection, air samples were obtained from the air using gelatine filters.

The second trial was conducted in an isolation unit that contained 6 pens (two rows of 3 pens: row A consisting of pens 1-3 and row B consisting of pens 4-6), housing 4 pigs each. The pens were build with wire fencing, so that in each row pigs in neighbouring pens could have direct contact. The rows were 2,5 meters apart of each other, allowing only contact through the air. Air flow was from row B to A. Two pigs in pen 6 were inoculated with the Paderborn strain, and the transmission to the other pens was studied.

Virus detection was carried out by virus isolation (VI; infectious virus) and PCR (viral RNA).

Results

In the first trial, all contact pigs became infected, except for the contacts of the Zoelen inoculated pigs. Infectious virus and viral RNA were detected in the air of rooms housing the pigs infected with the Paderborn and Brescia strains, but not in the room housing the pigs infected with the Zoelen strain. It was observed that the higher the dose or virulence of the virus strain, the sooner virus could be detected in the air samples. The amount of virus in the air correlated with the number of infectious pigs.

In the second trial, the between-pen transmission (row B) occurred with a delay of 11 days from pen 6 to 5 and another 7 days from pen 5 to 4. This transmission occurred almost certainly through direct contacts. The transmission through the air (to row A) was very efficient. The first few infections in row A occurred when all 4 pigs in pen 6 were infectious, expanding quickly when another 2 pigs from pen 5 became infectious. All pigs in row B became infected before they were able to infect each other by direct contact. The moments of detectable virus concentrations in the air matched the moment of infections in row A.

Discussion

This is the first study describing the successful detection of CSFV in air samples originating from infected pigs. Mainly pigs inoculated with the Brescia and Paderborn strain excreted detectable amounts of virus in the air. For pigs infected with the Paderborn strain, it was shown that transmission through the air, over a short distance, was very efficient and occurred when approximately 4-6 pigs were excreting virus. This transmission route could therefore play a role during outbreaks within pig units. A possible role of airborne transmission between herds still needs to be investigated further.

# ORAL: MONITORING OF THE FISH VIRAL DISEASES IN POLAND IN 2004-2008- FACTORS PREDISPOSING TO DISSEMINATION OF INFECTIOUS AGENTS

MATRAS, MAREK [1]; ANTYCHOWICZ, JERZY [1]; BORZYM, EWA[1]; REICHERT , MICHAŁ[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: monitoring, viral diseases

Frequency of VHS, IHN, IPN, SVC and KHV infection outbreaks in the last five years and the locations of infected fish farms in Poland were presented and discussed.
Two IHN cases in rainbow trout (Oncorhynchus mykiss) were detected in Poland for the first time and hitherto unrecognized rhabdovirus was isolated from grayling (Thymallus thymallus).
The factors predisposing the spread of fish viral diseases in Poland a specially the causes of VHS and KHV infection were analyzed.
The following factors contribute mainly to KHV introduction and spread in Poland:
• Uncontrolled movement of live koi carp in EU countries
• Stocking koi carps with carps reared to consumption together in production ponds
• Propagation of crossbred between ordinary carp and koi carp
• Lack of realistic long terms programmes for the KHV control in traditional large carp farm environment
• Lack of any effective legislation regulating movement of live carp and koi carp between the farms which didn't have status free of KHV
The following factors contribute mainly to growth of the number of VHS cases in Poland:
• Uncontrolled movement of live rainbow trouts between the infected regions and the regions of unknown epizootic status
• Negligence in realization of the long term VHS and IHN eradication programmes
• Lack of the regions and farms with recognized VHS and IHN free status
The growth of dynamics of viral fish disease incidences in Poland was determined. The results of performed monitoring showed that VHSV and KHV infections are the main epidemiological problems in Poland fish farms.
The following conclusions were made:
• The realization of the long term VHS, IHN and KHV infections is urgent to decrease the spread of these diseases
• The geographic information system (GIS) should be applied in the near future for efficient monitoring of the important fish viral diseases and for tracking the source of infection

# ORAL: HIGHLY MOBILE PATHOGENS AND THEIR EARLY DETECTION- POSSIBILITIES AND CONSTRAINTS OF AVIAN INFLUENZA SURVEILLANCE IN WILD BIRDS

WILKING, HENDRIK[1]; ZILLER, MARIO[1]; HARDER, TIMM C[1]; STAUBACH, CHRISTOPH[1]; CONRATHS, FRANZ J[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: Avian Influenza Monitoring Process control

Background: Monitoring wild birds, especially migratory birds, for pathogens is difficult. When cases of avian influenza are detected, it is often hard to assess the true number of affected animals and to estimate the prevalence accurately. When the large number of susceptible bird species is taken into account, it becomes obvious that a profound analysis of these data is a challenge for biometricians and veterinary epidemiologists.
Methods: For the analysis of surveillance data, we propose an evaluation model for estimating confidence intervals for (i) prevalence calculations in outbreak situations or (ii) the absence of disease in certain time intervals for certain regional units in Germany. For these point estimates, the upper confidence limits (UCL) where estimated. By introducing a scheme of species weighting, the surveillance system can be evaluated in terms of effectiveness of the birds sampled under the respective conditions.
Results: Prevalence estimates obtained during outbreak situations in wild birds in Germany (e.g. at the Baltic Sea in 2006) were subject to a reporting bias and distorted due to missing data. For most places and most times the UCL did not fall below 10%. Only intensive monitoring between November 2006 and June 2007 at the Baltic Sea led to reliable confidence limits over a long period. Between the outbreaks of wild swans in Nuremberg and in domestic ducks in Middle Franconia in 2007, the UCL varied around 25%. Different UCLs for different species selection scenarios were compared and used to optimise the sampling, leading to higher confidence in the surveillance system regarding virus prevalence and virus transmission activity.
Conclusion: The approach developed in this study may prove useful for the detection of time intervals, geographical units and population subgroups where monitoring is insufficient to detect a pathogen with low prevalence. In the future, it may be possible to monitor wild birds in a risk-based approach and to target specific animals with a better chance of detecting avian influenza viruses in the wild bird population.

# ORAL: QUANTIFYING FOOT-AND-MOUTH DISEASE AND SWINE VESICULAR DISEASE VIRUS TRANSMISSION PARAMETERS; COLLABORATIVE OUTPUT OF EPIZONE WP 6.3

EBLÉ, P.[1]; HAGENAARS, T.[1]; DEKKER, A.[1]; COX, S.[2]; BARNETT, P.[2]; ZHANG, Z.[2]; GORIS, N.[3]; DE CLERCQ, K.[4]; BELLINI, S.[5]; ALBORALI, L.[5]; ALEXANDERSEN, S.[6]; TJORNEHOJ, K.[7]; YIN, H.[8]; WOOLHOUSE, M.[9]; DE JONG, M.[10]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]; INSTITUTE FOR ANIMAL HEALTH (IAH)[2]; OKAPI SCIENCES[3]; VETERINARY AND AGROCHEMICAL RESEARCH CENTRE (VAR)[4]; ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL' EMILIA ROMAGNA (IZSLER)[5]; NATIONAL CENTRE FOR FOREIGN ANIMAL DISEASE[6]; NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[7]; LANZHOU VETERINARY RESEARCH INSTITUE (LVRI)[8]; UNIVERSITY OF EDINBURGH[9]; WAGENINGEN UNIVERSITY AND RESEARCH CENTRE[10]

Key words: foot-and-mouth, swine vesicular disease, transmission, modelling, EPIZONE

Foot-and-mouth is the disease most feared by livestock holders and veterinarians and since the 2001 outbreaks in Europe the need to more readily quantify viral transmission dynamics and transmission parameters has been recognised as fundamental for decision makers and modellers. The main goal of EPIZONE WP 6.3 is to quantify transmission parameters of FMDV and SVDV, which are needed not only to quantify the population dynamics of the viruses but also to estimate the effectiveness of intervention measures (e.g. vaccination). From several labs working on FMDV and/or SVDV, data from past experiments and/or field data are being inventoried and mathematical models are being used to estimate transmission parameters. The partners providing data are introduced to the use of these mathematical models. Overall, the group tries to define gaps and needs concerning transmission data on FMDV and SVDV with a view to collaborating on future experimental work.
Here we report on the output of WP 6.3. Using data from CVI, IAH, VAR-CODA, DTU-Vet, LVRI and IZSLER within-pen transmission parameters of pigs, cattle and sheep have been estimated. Besides the within-pen within-species, we have also estimated within-pen between-species transmission parameters. Apart from the parameters obtained by this way, the analyses of 'old' experiments using these newly developed techniques can also replace, reduce and refine future transmission experiments and as a consequence minimise animal suffering for research purposes. As a sequel of the analysis of field data of an SVDV outbreak, a questionnaire on bio-security will be established that will be used to make an inventory of the bio-security levels of pig-farms.
Another output of the WP is the transfer of knowledge. The mathematical methods that are being used to estimate transmission parameters were introduced to the WP members via two reports and we aim to provide a course on 'The design and analysis of transmission experiments' that will be open for all EPIZONE members. The contents of both reports and course will be presented.

Acknowledgements: We wish to thank all colleagues who shared their knowledge and/or information and collaborated with us. This work was supported by the EU Network of Excellence, EPIZONE (Contract No FOOD-CT-2006-016236).

# ORAL: IN VITRO-INDUCED HPAIV H5N1 ESCAPE MUTANTS: IN VITRO AND IN VIVO CHARACTERIZATION

KALTHOFF, DONATA[1]; HOEPER, DIRK[1]; BEER, MARTIN[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: avian influenza escape H5N1

Incomplete immunity after vaccination of poultry with inactivated avian influenza (AI) vaccines enables the occurrence of so-called "escape mutants" of circulating AIV which are characterized by a reduced vulnerability to vaccine-specific neutralising antibodies.
By propagating HPAIV H5N1 fifty fold on cell cultures (MDCK) in the presence of a neutralising antiserum from H5N2-immunized and H5N1-challenged chicken, the field situation in endemic outbreak regions was mimicked and an escape mutant generated (H5N1_P50esc). In parallel, a control virus (H5N1_P50) was produced by passaging the ancestor virus fifty times on MDCK cells without an antiserum. All newly generated H5N1 viruses were characterized by sequencing, growth analysis, determination of hemagglutination and neutralisation characteristics, and investigation of the intravenous pathogenicity index (IVPI) in chicken. The IVPI was determined for H5N1_P50 and H5N1_P50esc as well as for two intermediate mutants (H5N1_P18esc and H5N1_P30esc) after inoculation of 10⁵ TCID50/animal.
All mutants were attenuated in comparison to the ancestor virus, demonstrating a decline of the IVPI from about 2.9 (ancestor virus, high pathogenicity) to 1.2 (H5N1_P50; high pathogenicity) and 0.3 (H5N1_P50esc, low pathogenicity). In addition, transmission to naïve chicken was demonstrated for the control virus (H5N1_P50), whereas sentinel chicken in contact to H5N1_P50esc-infected animals remained uninfected. Full genome sequencing results (454/Roche) as well as the changed growth, neutralisation and hemagglutination characteristics will be presented. In conclusion, in vitro-induced escape from neutralizing activity resulted in several attenuated H5N1 mutants with unique characteristics. Finally, the use of "in vitro-escape" as a model system will be discussed.

# ORAL: NOVEL TRENDS TO COMBAT THE TRANSBOUNDARY ANIMAL DISEASES WORLDWIDE. ACHIEVEMENTS AND DISSEMINATION OF THE LAB-ON-SITE PROJECT OF THE EC

BELÁK, SÁNDOR[1]; UTTENTHAL, ÅSE[2]

NATIONAL VETERINARY INSTITUTE (SVA)[1]; NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[2]

Key words: TAD, OIE-listed diseases, diagnostic, dissemination

The highly contagious transboundary animal diseases (TADs) are regularly occurring and re-occurring on various continents, causing severe losses. This indicates the urgent need for the development of powerful, robust and high capacity diagnostic methods to identify the causative agents very rapidly. The LAB-ON-SITE project of the EU was run between 2004-2008, aiming the development of improved "first line" diagnostics that can be used by veterinarians in the field with development of robust and simple nucleic acid detection methodologies that can be used in local laboratories.

The targeted ten highly infectious TADs were: foot-and-mouth disease (FMD), swine vesicular disease (SVD), vesicular stomatitis (VS), classical swine fever (CSF), African swine fever (ASF), bluetongue (BT), African horse sickness (AHS), Newcastle disease (ND), highly pathogenic avian influenza (HPAI) and Swine influenza (SI).

The main scientific achievements of the project are summarized by the Consortium in the recent article written by Rodriguez-Sanchez et al. (2008).

Dissemination of results had a high priority; Workpackage 9 on "Transfer of knowledge to EU member and candidate countries, as well as to the third countries" was assigned to this task. To assure proper dissemination the project was prolonged. The importance of scientific publications had high priority but in addition we aimed at more popular presentations, this resulted in:

a) The methods were introduced in diagnostic laboratories and internationally harmonized, following OIE regulations;
b) A web-site: www.labonsite.com was created, it is regularly updated;
c) 19 new SOPs distributed to diagnostic laboratories many of them on a CD;
d) Booklets distributed in the IAEA network;
e) Workshops in Central and East Europe, as well as in the Far
f) Technology transfer to the veterinary community, to animal health authorities and to stakeholders;
g) Technical support, exchange visits or courses, all partners visited each other for teaching or training purposes;
h) Publication of 21 articles in international peer-reviewed journals, 41 proceedings, 21 oral presentations and 7 semi popular publications in a variety of languages, such as English, Danish, Spanish, Swedish and Hungarian. More publications are in progress.

By providing novel, highly sensitive, specific, high-throughput and robust methods for central institutes, for simply equipped field laboratories and for the on-site diagnosis in the field, the project had a strong contribution to the improved diagnosis and control of the devastating TADs worldwide. Herewith, the coordinator and the deputy coordinator of LAB-ON-SITE are presenting the achievements and dissemination of the project to the Network of Excellence EPIZONE in order to disseminate results and discuss the future improvement of strategies.

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# ORAL: DEVELOPMENT OF A DNA MICROARRAY FOR SIMULTANEOUS DETECTION AND GENOTYPING OF LYSSAVIRUSES

DASTJERDI, A[1]; GURRALA, R[1]; JOHNSON, N[1]; NUNEZ-GARCIA, J[1]; GRIERSON, S[1]; STEINBACH, F[1]; BANKS, M[1]

VETERINARY LABORATORIES AGENCY (VLA)[1]

Key words: Rabies, microarray, lyssavirus, Rhabdoviridae

The lyssavirus genus of the Rhabdoviridae family of viruses includes 7 genotypes and several non-assigned isolates. The source of lyssavirus infections is diverse with numerous reservoirs in a wide geographical area. In many parts of the world reservoir hosts can potentially be carrying one of several lyssavirus strains and possibly new divergent isolates await discovery. Accordingly, generic detection methods are required to be able to detect and discriminate all lyssaviruses and identify new divergent isolates. Here we have allied a sequence-independent amplification method to microarray to enable simultaneous detection and identification of all lyssavirus genotypes. To do so, lyssavirus RNA was converted to cDNA and amplified in a random PCR, labelled and hybridized to probes on the microarray chip before being statistically analyzed. The probes were to a 405bp region of the relatively conserved N gene. Here we demonstrate a microarray capable of detecting each of the seven lyssavirus genotypes. The random amplification of lyssavirus RNA and the numerous oligonucleotide probes on the microarray chip offer the potential to detect novel lyssaviruses.

# ORAL: QUALITY OF VETERINARY IMPORT RISK ANALYSIS STUDIES

DE VOS, CLAZIEN[1]; CONRATHS, FRANZ[2]; ADKIN, AMIE [3]; HALLGREN, GUNILLA[4]; PAISLEY, LARRY[5]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]; FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[2]; VETERINARY LABORATORIES AGENCY (VLA)[3]; NATIONAL VETERINARY INSTITUTE (SVA)[4]; NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[5]

Key words: animal health, audit form, import risk analysis, quality

Introduction

The application of import risk analysis has increased enormously since the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) of the World Trade Organisation (WTO) was implemented in 1995. According to this agreement, imports can only be refused when they pose a threat to the plant, animal, or human health in the importing country and this should be proven by risk analysis. Guidelines for import risk analysis concerning animal health issues were published by the Office International des Epizooties (OIE). However, not all import risk analysis studies (IRAs) adhere to these guidelines and the quality of the analyses performed is variable. The aim of this study was to obtain a general impression of the overall quality of IRAs by using a standardised audit form. Questions addressed were:

- Do different types of IRAs differ in quality?
- Are some parts of the IRAs of higher quality than others?
- How robust and objective is the method we used for reviewing?

Material and methods

An audit form was developed consisting of six sections, addressing (a) risk question and scope of the analysis, (b) risk assessment, (c) quantitative model if applicable, (d) data used, (e) risk communication, and (f) reporting. Each section consisted of several questions that could be answered using a drop-down list. Credits were given for each question, depending on the answer chosen. The maximum score for a qualitative risk analysis was 150 credits, whereas a quantitative risk analysis had a maximum score of 200 credits. These maximum values were used to calculate the relative score (between 0 and 1) of each IRA.

A total of 16 IRAs were reviewed of which nine were qualitative and seven were quantitative. Each IRA was reviewed by three different persons and thus had three (possibly different) scores. Mean values and standard deviations were calculated for comparison purposes.

Results

Preliminary results indicate that the quality of the IRAs differs considerably. The highest mean score obtained was 0.84, whereas the lowest mean score was 0.48. No significant differences were observed between qualitative and quantitative risk assessments. IRAs published in peer-reviewed journal papers had, on average, higher scores than those published in reports only (P=0.05). Sections with relatively high scores were (b) risk assessment, (c) quantitative models, and (d) data used. These sections can be considered the 'essential elements' of risk assessment. All other sections involve, to some extent, communication on risk assessment and its results, which is apparently more difficult to carry out. Not all audit results were consistent across reviewers. Ranges in scores per IRA were considerable with an average difference between minimum and maximum scores of 0.19. This might also be due to a reviewer bias. Comparing scores across reviewers, it is evident that some tended to give higher scores than others.

Conclusion

Despite international guidelines, IRAs are relatively heterogeneous with respect to quality. Quantitative IRAs do not necessarily score better than qualitative IRAs. Assessing the quality of IRAs using a standardised audit form is a transparent method to rate the quality of IRAs, although not objective, i.e., different reviewers end up with different scores.

# ORAL: ESTABLISMENT OF A BLUETONGUE VIRUS INFECTION MODEL IN MICE TO EVALUATE VACCINATION STRATEGIES

CALVO-PINILLA, EVA[1]; RODRIGUEZ-CALVO, TERESA[1]; ANGUITA, JUAN[2]; SEVILLA, NOEMÍ[1]; ORTEGO, JAVIER[1]

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[1]; VETERINARY UMASS[2]

Key words: Murine model BTV vaccination

Bluetongue (BT) is a non-contagious, insect-transmitted disease of certain breeds of sheep and some species of wild ruminants caused by bluetongue virus (BTV). A laboratory animal model would greatly facilitate the studies of pathogenesis, immune response and vaccination against BTV. Herein, we show that IFNAR(-/-) adult mice are highly susceptible to BTV-4 and BTV-8 infection when the virus is administered intravenously. Disease was characterized by ocular discharges and apathy, starting at 48 h post-infection and quickly leading to the death of the animals within 60 h of inoculation. Infectious virus was recovered from the spleen, lung, thymus, and lymph nodes (popliteal, inguinal, mediastinal, and mesenteric). In addition, a lymphoid depletion in spleen and thymus, and severe pneumonia was observed in the infected mice. Furthermore, IFNAR(-/-) adult mice immunized with a BTV-4 inactivated vaccine showed the induction of neutralizing antibodies against BTV-4 and complete protection against a lethal dose of this virus. The data indicate that this mouse model may facilitate the study of BTV pathogenesis, and the development of new effective vaccines for BTV.

# ORAL: INVESTIGATION OF THE EPIDEMIOLOGY OF AVIAN INFLUENZA IN WILD BIRDS AT LAKE CONSTANCE – A TRI-NATIONAL PROJECT OF GERMANY, SWITZERLAND AND AUSTRIA

BRUNHART, IRIS[1]; SCHWERMER , HEINZPETER[1]; BAUMER, ANETTE[2]; WILKING, HENDRIK[3]; RENNER, CHRISTIANE[4]; WASTLHUBER, ULRIKE[5]; GRIOT, CHRISTIAN[2]; STÄRK, KATHARINA[6]

FEDERAL VETERINARY OFFICE[1]; INSTITUTE OF VIROLOGY AND IMMUNOPROPHYLAXIS[2]; FEDERAL RESEARCH INSTITUTE FOR ANIMAL HEALTH[3]; MINISTRY OF FOOD AND RURAL AREAS[4]; STATE MINISTRY OF ENVIRONMENT AND PUBLIC HEALTH[5]; ROYAL VETERINARY COLLEGE[6]

Key words: avian influenza A, Lake Constance, surveillance programs

It is generally accepted that wild birds play an important role in spread avian influenza A viruses (AIV). When highly pathogenic avian influenza A H5N1 (HPAI H5N1) arrived in Europe in 2005, it was suggested that wild birds were involved in the propagation of this virus. In early 2006, HPAI H5N1 was detected in 32 bird samples at Lake Constance. In the context of this outbreak and the fact that the ecology and epidemiology of H5N1 in wild birds was largely unknown the research program "Constanze" was initiated to investigate AI infections in wild birds.
The objective of this paper is to provide a descriptive analysis of avian influenza in wild birds examined in the region of Lake Constance for a period of two years starting in September 2006 and to compare the performance of different surveillance methods in relation to bird species. The data analyzed was derived from birds found dead in the course of passive surveillance as well as from live feral birds tested within "Constanze" or other programs. Thus, a total of 2,003 samples from predominantely waterbirds resulted in the isolation of AIV from 45 samples of different subtypes but not HPAI H5N1 (2.24%).
1,482 samples were of the order Anseriformes and contained 42 positive samples (2.91%) found in mute swans, common teals and mallards. Three positive samples were derived from coots belonging to the second most tested order of Gruiformes (1.7%). Nevertheless, the small number of positive samples does not allow any conclusion on the potential role of particular species as carriers or shedders of AIV. 80% of the data came from active monitoring whereas 20% were derived from passive surveillance. All but two positive samples originated from birds tested within active monitoring i.e. caught birds tested alive or hunted birds tested dead. In an explanatory univariate test, active monitoring was shown to be more effective than passive surveillance for the detection of AIV infections in water birds. However, this observation does not necessarily pass a multivariate analysis. All positive samples were found between February 2007 and March 2008. Compared to a Poisson distribution, some months showed a significantly increased disease rate for AIV, suggesting common risk factors or more likely sampling bias for the different subtypes.
The temporal clustering of AIV infections leads to the hypothesis that LPAI viruses were introduced to the area of Lake Constance without persistence. Nevertheless, further analyses are needed to understand the spatiotemporal dynamics of these LPAI cases. Most of these infections were detected due to active monitoring rather than passive surveillance. As it was not possible to identify particular bird species with an increased susceptibility for AIV, all waterbirds should be targeted.

# ORAL: APPROACHES TO DIVA ASSAYS FOR BLUETONGUE DIAGNOSTIC

RANZ, A[1]; LÓPEZ, L[1]; RUEDA, P[1]; PÉREZ, T[1]; VELA, C[1]; SANZ, AJ[1]; VENTEO, A[1]

INMUNOLOGÍA Y GENETICA APLICADA S.A.[1]

Key words: DIVA, BTV

Introduction and Objectives

There are several commercial assays detecting antibodies specific of BTV, nevertheless, propose of vaccination at some countries of EC makes necessary to differentiate between vaccinated and infected animals. INGENASA has study two approaches to develop DIVA assays: a dual immnuoenzimatic assay based on NS3 and VP7 recombinant proteins and an indirect assay based on the use of NS1's peptides synthesized by "pepscan". On the other hand, an immunochromatographic assay of rapid detection has been developed to know "in situ" the situation of herds regarding presence of BTV's antibodies on serum and milk samples.

Material and Methods

• DIVA assay based on NS1: 15 amino acids overlapping peptides representing the complete sequence of NS1 were synthesized and checked as antigen in indirect ELISA using 20 sera of vaccinated and infected animals.
• Dual competition immunoassay: Recombinant VP7 and NS3 proteins were used for coating and two Monoclonal Antibodies specific of these proteins, as conjugates. The assay was evaluated with samples from two different vaccination/challenge studies (31 vaccinated and 8 not vaccinated). These animals were bled at different days post vaccination and post challenge. For specificity, 466 negative field samples were analyzed.
• Direct Immunochromatographic assay: in which BTV's VP7 recombinant protein is both adsorbed to a NC membrane and used as conjugate. To evaluate the performance of the assay, 48 negative sera and 52 positive sera (21 from vaccinated animals, 16 from experimentally infected cattle and sheep and 15 from naturally infected sheep) were analyzed. 137 individual milk and 3 tanks from different EC areas were used in addition.

Results

• Regarding DIVA assay based on NS1, with the data obtained, two peptides have been selected.
• Dual Competition ELISA showed to be able to distinguish among infected and vaccinated animals, within a period of 20 to 100 days post vaccination, when NS3 free vaccines are used. In these cases there is a clear difference regarding the response to NS3 (infected animals) and to VP7 (vaccinated and infected animals). After this days, the amount of antibodies specific of NS3 in infected animals decrease to disappear, making difficult the differentiation from vaccinated animals.
• In this study, IC assay showed 100% specificity using negative sera. Results for sera from vaccinated animals showed IC more sensitive than competition ELISA. Regarding infected animals sera, IC showed 100% correspondence with cELISA. 129 of 137 milk samples gave coincident results with cELISA.

Discussion

BTV situation requires all tools available in order to control it. Until now, ELISAs detecting antibodies has been of big help for this aim. Nevertheless, the fact of including vaccination in several countries of EC makes these assays poorly useful because of their incapacity to differentiate between vaccinated and infected animals. The Dual competition assay developed makes it possible among days 20 and 100 post vaccination, in which the titre of antibodies specific of NS3 is detectable. Results obtained have indicated that it is absolutely necessary for vaccines to be free of NS3. Regarding NS1 DIVA assay, two peptides have been selected for further studies with collections of sera from vaccinated and infected animals.
On the other hand, results obtained with the IC assay show that it is at least as sensitive as the ELISA. The advantage of IC led in its easy handling, when a rapid and "in situ" knowledge of the situation of a particular herd, is necessary.

# ORAL: MAPPING THE EFFECTS OF CLIMATE CHANGE ON BLUETONGUE TRANSMISSION IN EUROPE

MATTHEW, BAYLIS[1]; GUIS, HELENE[1]; CAMINADE, CYRIL[1]

UNIVERSITY OF LIVERPOOL[1]

Key words: bluetongue, climate change, risk, emergence, model

Bluetongue (BT) is an arboviral disease of ruminants which began emerging in Europe in 1998 and has subsequently caused an unprecedented series of epizootics of major economic consequence. Two distinct epidemiological patterns underlie this emergence: the northward expansion of the Afro-Asian midge Culicoides imicola in southern Europe and the involvement of indigenous European Culicoides vector species in northern and eastern Europe. This emergence, particularly in southern Europe, has largely been attributed to climate change, although no studies have yet assessed and quantified this link.

In order to evaluate the effects of recent and future climate change on the distribution of BT in Europe, we have integrated state-of-the-art series of observed and simulated climate datasets with climate-sensitive epidemiological models of the transmission risk of bluetongue. Transmission risk is here defined by the basic reproduction number (R0) of BT and in our model it is influenced by the dependence of virus transmission on temperature and of vector abundance on temperature and rainfall. Our unique approach allows the quantifying and mapping of R0 throughout Europe under past, present and future conditions simulated using several different climate models, with outputs in terms of mean anomalies and future trends.

R0 was computed for a population of two hosts, cattle and sheep. Both the exotic (C. imicola) and indigenous (Obsoletus group) vector distributions were included.

Climatic data for recent past (1961-2000) and future time slices up to 2050 were provided by the ENSEMBLES European project at a spatial scale of 25*25 km. For the recent past, improved regional climate simulations were produced by running a subset of ten regional climate models with the most realistic boundary conditions (ERA40 reanalysis) and external forcing. For the future conditions, simulations were carried out by running the regional climate models forced by the SRESA1B scenario from the Intergovernmental Panel on Climate Change.

By comparing past and present bluetongue outbreaks with R0 model outputs, we show that although there are uncertainties relative to the estimation of some parameters, the model is coherent with the observed epidemiological situation. Most importantly, R0 models support the fact that recent observed climate has facilitated the northward extension of C. imicola in Southern Europe whereas in Northern Europe climate has had little influence on the vector distribution and mainly influenced the other parameters of transmission (biting rate and virus replication). Future trends suggest that although the transmission risk will globally increase in Europe, the increase should be more important in Northern Europe.

# ORAL: CLASSICAL SWINE FEVER – ERADICATION IN THE AMERICAS, AND COOPERATION WITH THE OIE AND EUROPEAN UNION REFERENCE LABORATORY (OIE-EURL)

GREISER-WILKE, IRENE[1]; MOENNIG, VOLKER[1]

EURL FOR CLASSICAL SWINE FEVER[1]

Key words: classical swine fever; eradication programs; Cuba; Colombia

Pork is one of the most important nutritional protein resources for people worldwide, and production has and will increase in the future. Classical Swine Fever (CSF), one of the most economically important viral infectious diseases of pigs can severely affect swine production, being epizootic in parts of Europe and enzootic in Asia and South and Central America, whereas Australia, USA and Canada, have been free of CSF for more than 30 years. In endemic regions one of the main problems for control of CSF is the high number of backyard pig holdings. In South America and the Caribbean, a Plan to Eradicate CSF from the Americas by 2020 was started. It was the result of a discussion between specialists in the disease and various official Veterinary Services of the region taking into consideration their experiences with control and eradication of CSF during a workshop organized for the purpose by FAO and the Agriculture and Livestock Service of Chile in Santiago, in 1999 and in Costa Rica, 2001 (http://www.fao.org/regional/Lamerica/prior/segalim/animal/ppc/plan/orga.htm). As one of the consequences of this plan, different countries are and have updated their diagnostic skills, and have been or are preparing to have their diagnostic laboratories accreditated. In this context, collaborations of the OIE-EURL with Cuba (2005) and Colombia (2008) were started. With Cuba, a twinning project is under way.

Duties of the OIE-EURL include the coordination of diagnostic methods, the training of experts in all fields of laboratory diagnosis with a view to harmonize diagnostic techniques, and the organisation of comparative tests (inter-laboratory comparison tests) of diagnostic procedures.

In Cuba, in spite of extensive vaccination CSF is enzootic. Besides a new plan for eradication of CSF (2006), considerable efforts are done to solve the problem, including the development of a new subunit vaccine and a pen-site test to detect CSFV specific antibodies. In the mean time, the National Center for Animal and Plant Health (CENSA) is recognized by the Food and Agriculture Organization of the United Nations (FAO) as a regional FAO-Collaborating Center for the preparedness on animal trans-boundary diseases in the Caribbean Region. CENSA is participating in the inter-laboratory comparison testing as organized by the OIE-EURL since 2005. CENSA also operates REDesastres (http:// www.censa.edu.cu/cedesap), a virtual community that disseminates information both from both national and international sources. The scientific community involved also includes colleagues from Argentina, Colombia, Costa Rica, Dominican Republic, Mexico, Peru and Venezuela.

The last CSF outbreaks in Colombia occurred in 2007. An eradication program involving vaccination with C-strain was initiated in the year 2000, and directed and advised by the Instituto Colombiano Agropecuario (ICA). Vaccination and animal tagging is performed in close cooperation with the Association of Colombian Pig Producers. Vaccination was stopped in 2005, but CSF re-emerged in regions with difficult access to the pigs. Here, vaccination efforts have been increased. Between 2008 and 2013 it is planned to declare regions in the central part of the country, where pig production is mainly industrial, free of CSF, and to implement protection zones around them. The ICA is the only laboratory performing CSF diagnosis in Colombia, and is working together with the OIE-EURL on the accreditation of the laboratory. The ICA is now also participating in the inter-laboratory comparison testing as organized by the OIE-EURL.

# ORAL: TREATMENT OF PIGS WITH THE ANTIPESTIVIRUS COMPOUND BPIP REDUCES TRANSMISSION OF CSFV TO UNTREATED PIGS

VRANCKEN, ROBERT[1]; HAEGEMAN, ANDY[1]; PAESHUYSE, JAN[2]; PUERSTINGER, GERHARD [3]; ROZENSKI, JOZEF [2]; WRIGHT, MATTHEW [4]; TIGNON, MARYLÈNE [1]; LE POTIER, MARIE-FRÉDÉRIQUE [5]; NEYTS, JOHAN[2]; KOENEN, FRANK[1]

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE (VAR)[1]; REGA INSTITUTE FOR MEDICAL RESEARCH, K. U. LEUVEN[2]; INSTITUTE OF PHARMACY, UNIVERSITY OF INNSBRUCK[3]; GILEAD SCIENCES[4]; AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[5]

Key words: Antivirals; pestivirus; classical swine fever virus; virus transmission

1.Objectives

Evaluate the effect of an antiviral treatment on virus transmission between (i) pigs experimentally infected with classical swine fever virus (CSFV) and treated with the antipestivirus compound 5-[(4-bromophenyl)methyl]-2-phenyl-5H-imidazo[4,5-c]pyridine (BPIP), and (ii) untreated pigs.

2.Materials and Methods

Large White pigs (n=4) received BPIP (75mg/kg/day) in food pellets for 15 consecutive days. One day after first administration, pigs were infected intramuscularly with 104.5 TCID50 of CSFV 'Wingene'. At 2 days post infection (dpi) untreated sentinel pigs (n=4) were placed in an adjacent pen allowing nose to nose contact. Animals were clinically observed for 33 (treated) or 40 days (contact) and blood sampled 2 to 3 times a week. The presence of infectious virus was examined using standard methods and viral RNA in blood and tonsils was examined using real-time RT-PCR.

3.Results

Treatment with BPIP resulted in a marked reduction of the period of viraemia and virus (genome) titres. Two out of four animals in contact with the treated seeder pigs developed a short transient infection and have a significant lower virus (genome) load than the positive control group (p=0.015). A third animal became positive on real-time RT-PCR with a very low genome load. Time until onset of viraemia of the positive control group by means of virus isolation was 15.75 ± 2.36 dpi (4/4 infected) and 20.00 ± 8.49 dpi for the sentinel pigs in contact with BPIP-treated seeder pigs (1/4 infected).

4.Discussion and conclusion

BPIP, an in vitro inhibitor of CSFV-replication (1, 2), significantly reduced the virus titre and the viral genome load in blood of CSFV-infected pigs. Virus load in viraemic contact animals was significantly decreased. Although the time of onset of viraemia between the groups of contact animals did not significantly differ (p=0.126), a tendency towards the reduction of virus transmission was observed. This exploratory trial can be regarded as a worst case scenario since sentinel animals were left untreated and is therefore an underestimation of the potential efficacy of the activity of BPIP on virus transmission. Taking into account the small interval needed between drug application and antiviral activity, a rapid intervention with an antiviral drug could be beneficiary for containing the infection and to reduce between-herd virus transmission.

5.Acknowledgements

This work was supported by the EU Network of Excellence, EPIZONE (Contract No FOOD-CT-2006-016236).

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# ORAL: TO REPORT OR NOT TO REPORT: A PSYCHOSOCIAL INVESTIGATION AIMED AT IMPROVING EARLY DETECTION OF AVIAN INFLUENZA OUTBREAKS

ELBERS, ARMIN[1]; GORGIEVSKI-DUIJVESTEIJN, MARJAN[2]; ZARAFSHANI, KIUMARZ[3]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]; INSTITUTE OF PSYCHOLOGY[2]; RAZI UNIVERSITY[3]

Key words: avian influenza; vigilance in reporting; psychosocial factors

The aim of this study was to identify bottlenecks and solutions to overcome these barriers with the ultimate aim to facilitate early detection of Avian Influenza (AI) outbreaks. Focus group sessions were held with policy makers from the veterinary authorities, and representatives of veterinary practitioners and poultry farmers. Personal interviews with a small group of poultry farmers and practitioners were held to check proposed bottlenecks and solutions. An electronic questionnaire was mailed via an e-mail newsletter to members of a poultry farmer organization and posted for three weeks on the website of the Royal Dutch Veterinary Association. The questionnaire was subdivided into four sections. Section a) asked when and under what conditions one would report a clinically suspect situation. Section b) asked about feelings and (economic) consequences one expected after reporting a clinical suspicion. These questions were formulated both for the case that, retrospectively, clinical signs would indeed turn out to be caused by AI (true positive), as well as the situation that in retrospect it would become clear that this was not caused by AI (false positive). Section c) asked about barriers for reporting; and d) about opinions on national regulation explaining when and how to report a clinically suspect situation. Finally, the questionnaire did not just probe into possible bottlenecks, but also possible solutions to break down the barriers.

A total of 33 poultry farmers and 334 veterinary practitioners responded. After triangulating the responses of veterinary authorities, veterinary practitioners and poultry farmers, six themes emerged across all groups: 1) lack of knowledge and uncertainty about clinical signs of AI; 2) guilt, shame and prejudice; 3) negative opinion on control measures; 4) dissatisfaction with post-reporting procedures; 5) lack of trust in government bodies; 6) uncertainty and lack of transparency of reporting procedures.

The following solutions to facilitate early detection of AI were put forward by poultry farmers and veterinary practitioners: 1) possibility to submit blood samples directly to the national reference laboratory (NRL) to exclude AI in a clinical situation with non-specific clinical signs, without isolation of the farm and free of charge for the individual farmer; 2) select post-mortem submissions with a specific range of preliminary diagnoses from veterinary practices to be send to the NRL for antigen testing (PCR) to exclude AI as a possible cause for the disease problems in these poultry flocks; 3) decrease social and economic consequences of reporting AI, for example by improving the public opinion on first reports; 4) better schooling of veterinary officers to deal with emotions and insecurity of farmers in the process after reporting; 5) better communication of rules and regulations, where to report, what will happen next; 6) there is a need for continuous training of poultry farmers and veterinary practitioners with respect to recognizing clinical signs associated with AI; besides training, this can partly be facilitated by an up-to-date website with information and visual material (photo and video) of the clinical signs of AI.

# ORAL: DEVELOPMENT OF A MULTIPLEX RT-PCR TECHNIQUE FOR DIFFERENTIATION OF FMDV SEROTYPES IN TURKEY

SAREYYUPOGLU, BEYHAN[1]

FOOT AND MOUTH DISEASE INSTITUTE (SAP)[1]

Key words: Multiplex RT-PCR, FMDV, serotypes, Differentiation

Beyhan Sareyyüpoglu *, İbrahim Burgu**
*FMD Institute, Ankara, TURKEY
**University of Ankara, Department of Virology, Ankara, TURKEY

Abstract
1. Introduction and objectives: FMDV has distinctive characteristics such as high mutation rate, persistence, quasi-species population structure, rapid replication, easy adaptation which cause difficulties in its diagnosis2.
In this study, novel FMDV genus specific and serotype specific (A and O specific) forward primers were designed and an original multiplex RT-PCR (mRT-PCR) was developed to differentiate FMDV serotypes in Turkey.
2. Materials and Methods: Totally, 272 samples were provided from different outbreaks in Turkey. Variable antigenic regions in VP1 were targeted for the design of type specific forward primers, and, 2AB region was targeted for a common FMDV genus specific reverse primer 1,7. Optimization of mRT-PCR were performed due to the previously reported suggestions 3,4,5,8.
3. Results: As a conclusion, the mRT-PCR strategy with one common reverse and two serotype specific forward primers was found to be successful to differentiate FMDV serotypes. Of the 272 field samples (tongue epithelium, heart) 124 (45.5 %) were found to be positive in FMDV ELISA (performed to detect serotypes, A and O), while, 211 (77.5%) were positive in mRT-PCR. Sensitivity and specificity of mRT-PCR was determined as %84 and % 95, respectively. Significant difference ($p<0.05$) was detected in the results of ELISA and mRT-PCR positivity.
4. Discussion: VP1 region has also been targeted for primer design in previous studies 3,6, nevertheless, it is difficult to compare the results of these previous studies and this recent study, since different protocols were used. As a common finding, higher positive results were obtained in mRT-PCR compared to ELISA. Analytical sensitivity of mRT-PCR was higher than the ELISA in the detection of either FMDV serotypes. Newly designed primers were found to be specific to regarding FMDV serotypes, and they did not amplify SWD, BVD-MD, BHV, BTV DNAs.
The novel mRT-PCR technique developed with the originally designed primers is a rapid and reliable method, providing a significant advantages compared to the ELISA. Investigation of ELISA negative samples with mRT-PCR as a second round test is suggested for the improvement of the diagnostic success.

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# ORAL: RISK MAPS FOR THE SPREAD OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN POULTRY

HAGENAARS, THOMAS[1]; BOENDER, GERT JAN[1]; BOUMA, ANNEMARIE[2]; NODELIJK, GONNIE[1]; ELBERS, ARMIN[1]; DE JONG, MART[3]; VAN BOVEN, MICHIEL[4]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]; FACULTY OF VETERINARY MEDICINE, UTRECHT UNIVERSITY[2]; WAGENINGEN UNIVERSITY AND RESEARCH CENTRE[3]; NATIONAL INSTITUTE FOR PUBLIC HEALTH AND THE ENVIRONMENT[4]

Key words: Avian Influenza, Emerging Zoonoses, Intervention Strategies, Transmission Dynamics, Epidemic Models

Devastating epidemics of highly contagious animal diseases such as avian influenza, classical swine fever, and foot-and-mouth disease underline the need for improved understanding of the factors promoting the spread of these pathogens. Here we present a spatial analysis of the between-farm transmission of a highly pathogenic H7N7 avian influenza virus that caused a large epidemic in The Netherlands in 2003. We developed a method to estimate key parameters determining the spread of highly transmissible animal diseases between farms based on outbreak data. The method allows for the identification of high-risk areas for propagating spread in an epidemiologically underpinned manner. A central concept is the transmission kernel, which determines the probability of pathogen transmission from infected to uninfected farms as a function of inter-farm distance. We show how an estimate of the transmission kernel naturally provides estimates of the critical farm density and local reproduction numbers, which allows one to evaluate the effectiveness of control strategies. For avian influenza, the analyses show that there are two poultry-dense areas in The Netherlands where epidemic spread is possible, and in which local control measures are unlikely to be able to halt an unfolding epidemic. The analyses provide an estimate of the spatial range over which highly pathogenic avian influenza viruses spread between farms, and emphasize that control measures aimed at controlling such outbreaks need to take into account the local density of farms.

Work published as: Boender GJ, Hagenaars TJ, Bouma A, Nodelijk G, Elbers ARW, et al. (2007) Risk maps for the spread of highly pathogenic avian influenza in poultry. PLoS Comput Biol 3(4): e71. doi:10.1371/journal.pcbi.0030071

# ORAL: GENETIC STABILITY OF PESTIVIRUS GENOMES CLONED INTO BACS

RASMUSSEN, THOMAS BRUUN[1]; REIMANN, ILONA[2]; UTTENTHAL, AASE[1]; BEER, MARTIN[2]

NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[1]; FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[2]

Key words: Pestivirus, BAC, Infectious clone, DIVA vaccine

Infectious cDNA clones are a prerequisite for directed genetic manipulations of pestivirus genomes to obtain attenuated pestiviruses designed as new modified live DIVA vaccine candidates against classical swine fever. However, the construction of new infectious pestivirus cDNA clones has been hampered due to the large size of the pestivirus genome and due to genetic instability of the cloned cDNA, which in combination with plasmid vectors tend to be unstable and deleterious in the bacterial host. Therefore, new strategies are needed to facilitate construction of stable infectious cDNA clones of pestivirus strains.

In a collaborative research project, between DTU Vet and FLI, on the establishment of genetically modified pestiviruses engineered specifically for the DIVA principle, we cloned a series of complete pestivirus genomes, obtained by full-length RT-PCR, directly into the bacterial artificial chromosome (BAC) vector "pBeloBAC11". This BAC vector provides a markedly higher stability of cloned sequences in E. coli compared to plasmids that form the basis for the existing pestivirus cDNA clones. In this study, two of the newly constructed BAC clones were analysed for genetic stability of the cloned pestivirus genomes to demonstrate the suitability of the BAC vector for harbouring pestivirus genomes. Two BAC clones, comprising the complete genomes of BDV Gifhorn (pBeloGif3) and CSFV Paderborn (pBeloPader10) were passaged 15 times in E.coli representing at least 360 bacteria generations. From 15th passage of the BAC clones, the entire 5′ and 3′ ends of the cloned genomes and parts of the open reading frame were sequenced and compared to the sequences of the parent BAC clones. The sequenced areas represent approximately 20 % of the cloned genome. No mutations were observed after the extensive passaging of the cDNA clones in the bacterial host, indicating a highly stable system for cloning and maintenance of complete pestivirus genomes.

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# ORAL: EVALUATION OF CSFV ANTIBODY ELISAS FOR THE DIFFERENTIATION OF INFECTED FROM VACCINATED ANIMALS

SCHROEDER, SABINE[1]; BLOME, SANDRA[2]; KOENEN, FRANK[3]; LOEFFEN, WILLIE[4]; RASMUSSEN, TANYA[5]; HAEGEMANN, ANDY[3]; UTTENTHAL, ASE[5]

UNIVERSITY OF VETERINARY MEDICINE HANNOVER (HVS)[1]; FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[2]; VETERINARY AND AGROCHEMICAL RESEARCH CENTRE (VAR)[3]; CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[4]; TECHNICAL UNIVERSITY OF DENMARK[5]

Key words: CSF, antibody ELISA, DIVA,

Classical swine fever (CSF) is one of the most important epizootic diseases of pigs. With few exceptions the Member States of the European Union (EU) are currently considered to be free of CSF in domestic pigs. However, the disease is still endemic in the wild boar populations of several European countries and outbreaks occurred recently e.g. in Germany, France, Hungary, Romania, Bulgaria, and the Slovak Republic.
Preventive vaccination is prohibited within the EU, but emergency vaccination can be part of the strategy in case of a contingency. Using conventional vaccines, differentiation of vaccinated from infected animals (DIVA) is not possible. Newly developed modified live marker vaccines allow a DIVA strategy based on the use of enzyme linked immunosorbent assay (ELISA) tests.
The aim of this study was to evaluate CSF virus (CSFV) Antibody ELISAs, commercially available in Europe, for their diagnostic sensitivity as well as for their potential in differentiating between infected and marker vaccinated animals. Two newly available ELISAs were included into the tests, the Priocheck® CSFV Erns ELISA, a special DIVA test, and the LDL Pigtype® CSFV Antibody ELISA.
An inter-laboratory comparison test with four EU national CSF reference laboratories and the EU reference laboratory participating was organized. Seven different CSFV antibody ELISA test kits, targeting distinct antibodies (against E2, Erns, NS3) were provided to the participating laboratories together with a set of 41 samples.
This set included the following, well characterized samples derived from animal experiments: CSFV antibody positive sera with low, medium and high titers, sera free of CSF antibodies, sera from pigs infected with pestiviruses other than CSFV (Bovine viral diarrhoea type I and II and Border disease virus), sera from pigs vaccinated with conventional vaccines (C-strain, GPE-), sera from pigs vaccinated with E2 subunit vaccine and recent 3rd generation marker vaccines (cp7E2Alf, cp7E2gif, pRiemsABCgif, FLc11, FLc9), as well as sera from pigs vaccinated and afterwards challenged with CSFV.
In addition each of the laboratories was asked to additionally test approximately 50 samples from their national pig herds, which were supposed to be negative and approximately 100 samples from DIVA experiments to assure a wide coverage of different serum samples.
Practicability, diagnostic sensitivity, specificity, repeatability, and reproducibility were calculated and conclusions were drawn on the feasibility of using existing Ab-ELISAs for DIVA testing. The results will be presented at the meeting.

Acknowledgement: This work was supported by the EU Network of Excellence, EPIZONE (Contract No FOOD-CT-2006-016236).

# ORAL: MICRO-ARRAYS AS A MOLECULAR IDENTIFICATION TOOL FOR POSSIBLE BTV-8 VECTORS

DE DEKEN, GILL[1]; DEBLAUWE, ISRA[1]; DE WITTE, KO[1]; MADDER, MAXIM[1]

INSTITUTE OF TROPICAL MEDICINE ANTWERP[1]

Key words: micro-arrays, Culicoides, bluetongue,

After the 2006 outbreak of bluetongue virus serotype 8 (BTV-8), Belgium started a national vector monitoring programme, coordinated by the animal health department of the Institute for Tropical Medicine Antwerp (ITMA). Results soon showed the importance of local vector species in the epidemiology of BTV-8. Based on virus extraction tests the most probable Culicoides vectors belonged to the C. obsoletus-complex (C. obsoletus, C. scoticus, C. dewulfi, C. chiopterus) and the C. pulicaris complex (C. pulicaris, C. punctatus, C. lupicaris, C. deltus, C. impuctatus, C. fagineus, C. newsteadi, C. grisescens).

Specimen (especially females) belonging to either of these complexes are often hard to differentiate through morphological features, which is still the most common used tool to identify Culicoides.

In order to gain certainty about the correct identification of these species, ITMA developed a molecular identification tool based on the use of micro-arrays.

Micro-arrays are small solid supports (in our case SAL-coated glass slides) onto which sequences of different genes (so called probes) are coated at specific locations.

Through hybridisation with DNA, RNA or PCR-products and fluorescent detection these probes can be brought to expression.

In our case species specific probes were designed based on the interspecies differences within the sequences of the Internal Transcribed Spacer 1 (ITS1 (rDNA)).

Those probes were tested on their uniqueness using a local BLAST search against a database of all available Gen Bank sequences. A total of 16 different probes were developed for 11 different species and 1 common probe for all Culicoides spp. taking into account GC-content and basepair length.

First the probes and hybridisation reaction for the C. obsoletus complex were optimised, as this is the complex with the highest abundance in Belgium.

The micro-array test for the C. obsoletus-complex was validated through a large in-house blind test and was also compared with other molecular tools during a ring trail organised by MEDREONET among several European laboratories. Further work focuses on specific probes to identify the C. pulicaris complex and other indigenous species.

# ORAL: A DECISION ANALYSIS MODEL FOR THE CONTROL OF FOOT AND MOUTH DISEASE

MINTIENS, KOEN[1]; NATH, MUNMUN[1]; HOUDART, PHILIPPE[2]; DE CLERCQ, KRIS [3]

VOSE CONSULTING[1]; FEDERAL AGENCY FOR THE SAFETY OF THE FOOD CHAIN[2]; VETERINARY AND AGROCHEMICAL RESEARCH CENTRE (VAR)[3]

Key words: Decision analysis, emergency vaccination, risk management

Foot and mouth disease (FMD) is a highly contagious disease causing high morbidity and mortality in livestock. FMD was eradicated from the European Union by the end of the 20th century but the 2001 FMD-epidemic in the UK has shown that a (re-)introduction of the virus in a naive population can have devastating consequences. Early detection of a (re-)introduction, efficient elimination of infected animals, and reduction of the population at risk are key elements for successful control of a FM-epidemic. Historically, reduction of the population at risk was only possible through culling and destruction of (apparently) healthy animals as emergency vaccination was not allowed. Recent developments in vaccines and differential diagnostics (NSP-tests) have made that the European Commission has accepted emergency vaccination as an alternative. Still emergency vaccination may not always be the best option, since it implies addition trade-restrictive measures.

The aim of this study was to evaluate the different alternatives for reducing the population at risk when controlling FMD-outbreaks. Decision analysis methods were used since a decision for the best alternative is based in multiple criteria and incorporates uncertainty. In general, an decision analysis comprises several steps: 1) identification of the decision situation and context, 2)identification of values and objectives, 3) identification of alternatives, 4) decomposition and modelling of problem structure, uncertainty and preferences, 5) choice of the best alternative, and 6) sensitivity analysis.

The context of the decision process was derived from the European Council Decision 2003/85/EC on Community measures for controlling FMD. By interviewing different stakeholders the main objectives of decision process were set as reduction of the population at ris by 1) destruction of as little animals as possible and 2) minimising economical losses. The decision alternatives where preventive culling and emergency vaccination to live. In addition, these alternatives were considered for the different susceptible livestock species. As a result of the sensitivity analysis it was found that best alternatived was mainly depending on the population density and structure, and the renderning and vaccination capacity. The influence of these parameters on the decision will be illustrated durin the presentation.

This study has shown that decision analysis models can be efficient risk managament tools to assist decision makers with motivating the best alternative for controlling FMD-outbreaks.

# ORAL: HIGHLY PATHOGENIC AVIAN INFLUENZA IN WILD BIRDS IN DENMARK 2006: SPACE AND TIME-SPACE CLUSTERING

WILLEBERG, PREBEN[1]; JORGENSEN, POUL H.[2]; PEREZ, ANDRES[1]; ALKHAMIS, MOHAMMAD[1]

CADMS, UNIVERSITY OF CALIFORNIA[1]; NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[2]

Key words: HPAI H5N1, epidemiology, occurrence, wild birds, clustering

The purpose of the study was to quantify the time-space clustering of H5N1 highly pathogenic avian influenza (HPAI) outbreaks in wild birds reported in Denmark in 2006.

Records from the epidemic were provided by the Danish Veterinary and Food Administration. Data included 45 cases of H5N1 HPAI infection in wild birds from a population of 6,424 tested samples. The 45 cases were confirmed by virus isolation (n=22) or by RT-PCR (n=23). The Poisson model of the space scan statistic was used to identify geographical regions at high risk of infection, assuming that the population at risk was the number of samples analyzed by the national reference laboratory (n=6,424). The maximum spatial extension of clusters was set to a radius of 40 km based on applicability of the results to conditions in Denmark. Significance of candidate clusters was estimated using a Monte-Carlo simulation process. The model was re-run, adjusting by the origin of the sample (dead birds or fecal samples) and by bird species sampled. Additionally, a time-space Poisson model was run to identify clusters of H5N1 HPAI-positive dead birds within the 718 dead birds submitted to the national reference laboratory from February 13 through April 28, which was the period in which positive results from dead birds (n=43) were obtained. The time-space model was re-run again, this time, adjusting by bird species.

Data and results were displayed using an interactive web-based system referred to as the FMD BioPortal and are publicly accessible at: http://fmdbioportal.ucdavis.edu (username: "Denmark"; Password: "avianflu").

An area at high risk (P<0.05) of H5N1 HPAI infection was consistently identified through the runs of the space and time-space analyses. The cluster was centered at 54.9 N, 10.0-10.4 E, depending on the specifications used to run the simulations. Adjustment of the model by the origin of the sample and by the bird species sampled resulted in reduction of the cluster size, of the observed-to-expected ratios, and of the log likelihood ratios.

Results suggest that the time-space clustering of H5N1 HPAI in 2006 in Denmark was partly related to the heterogeneous origin of samples and the uneven distributions of the bird species sampled with respect to time and space. Further analyses at the level of the Baltic Sea Region as well as integrated phylo-genetic evaluation of sequenced virus isolates might give additional insight into the epidemiological pattern observed during the regional 2006 epidemic of HPAI H5N1 in wild birds.

# ORAL: "DOUBLE CHECK – DOUBLE TUBE STRATEGY" IMPROVED SAFETY FOR THE DETECTION OF BLUETONGUE VIRUS GENOME IN SEMEN

HOFFMANN, BERND[1]; BEER, MARTIN[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: BTV, real-time RT-PCR, semen, internal control

In 2006 the Bluetongue Virus serotype 8 (BTV-8) was ascertained in the border region of the Netherlands, Belgium and Germany. In the last three years a massive spread of BTV-8 was observed and additional BTV serotypes threatened sheep-farming and cattle production. Therefore, also the infection of bulls has to be taken into consideration, and OIE regulations recommend the free-testing of animals and not of semen samples, although the risk of BTV transmission due to semen is very low. Nevertheless, improved diagnostic methods allow new options for a safe and sensitive BTV genome detection directly in semen. We compared different extraction methods in regard to effective and inhibition-free purification of BTV-RNA using spiked as well as naturally BTV-8-infected semen samples. A combined procedure using "Trizol reagent" for lysis, and the "RNeasy column" for final purification delivered the most qualified and suitable viral RNAs. Combined with a highly sensitive real-time RT-PCR (rRT-PCR) protocol for the detection of all 24 BTV serotypes in one tube, and a BTV-8 specific rRT-PCR with equal sensitivity in a second tube, very robust and reliable results could be produced concerning the detection of BTV-8 in naturally infected semen. For the control of successful RNA extraction and purification, two internal control systems were additionally integrated: (1) The pan-BTV rRT-PCR was combined with a control system based on an external heterologous in vitro RNA; (2) The duplex BTV-8 rRT-PCR co-amplified an internal control using the housekeeping gene beta-actin as a target sequence. This "double check – double tube strategy" including two independent internal control systems markedly improved the robustness and sensitivity of the rRT-PCR analyses and could be a prerequisite for the acceptance of direct testing of semen in the future.

# ORAL: BLUETONGUE SURVEILLANCE IN FINLAND 2007-2009

RIKULA, ULLA[1]; JAKAVA-VILJANEN, MIIA[1]; ELLONEN, LAURA[1]; KAARTINEN, LIISA[1]; KORPENFELT, SIRKKA-LIISA[1]; LAAMANEN, ILONA[1]; LAAKSONEN, SAULI[1]; AALTONEN, TAINA[1]

FINNISH FOOD SAFETY AUTHORITY EVIRA[1]

Key words: bluetongue, surveillance, Finland

Introduction

Before 1998 bluetongue (BT) was an exotic infection in Europe. From 1998 through 2005 five serotypes of BTV have emerged in the Mediterranean Europe. A new serotype 8 was first notified in the Netherlands and it caused outbreaks also in Belgium, Germany, Luxembourg and France in 2006. In 2007 BT became endemic in these areas, and it spread further northwards to Denmark and westwards to UK in 2007. Serosurveillance for BT was started in Finland in 2007. Following the spread of BTV in southern parts of Sweden an additional sampling of dairy herds was performed in autumn 2008. Furthermore, all imported ruminants and a sample of wild cervids has been tested for BT since 2007. The surveillance plans for 2009 have been updated.

Materials and methods

A total of 1 677 samples (340 herds) in 2007 and 2 624 samples (404 herds) in 2008 were taken at slaughter from animals of suckler cow herds situated in south-western parts of Finland, and tested for BTV antibodies. During this 2-year period, all 209 imported domestic ruminants, 193 samples from wild cervids; white-tailed deer (Odocoileus virginianus) n=126, roe deer (Capreolus capreolus) n=42 and moose (Alces alces) n=25, and a further 30 samples due to clinical suspicions were tested for BTV. Additionally, in November 2008 bulk milk samples from all dairy herds in Ahvenanmaa, in south-western and southern coast of Finland were tested for BTV antibodies. Sera and bulk milk samples were tested by competitive serum and indirect milk ELISAs (ID Vet. Montpellier, France). RT-PCR modified from Shaw et al. (2007) was used for virus detection. Surveillance of small ruminants is based on clinical monitoring.

Results

All 4 301 samples taken at slaughter tested negative. One of the 748 bulk milk samples taken in November 2008 tested positive, but further investigation of the herd showed it to be a false positive result on milk indirect antibody ELISA. Neither antibodies nor virus could be detected on individual samples taken from the herd. All samples from imported animals, herds with clinical suspicions and wild cervids tested negative.

Discussion

Based on the BT surveillance and infectious disease data, Finland is considered to be BT free. Entomological data is sparse, but some potential vector species seem to exist. The number of imported ruminants is extremely small, however, hundreds of thousands doses of bovine semen and hundreds of bovine embryos are imported annually. In 2009 surveillance for BT will be intensified. All dairy herds considered to be at the highest risk for wind-borne BT will be sampled monthly from June to November and samples from animals of suckler cow herds at the same area will be taken at slaughter. Ten percent of the dairy herds situated south to 65° N will be sampled twice to cover also the most densely populated areas. All imported ruminants will be tested in quarantine both for the antibodies and virus. The authorities recommend to import ruminants only during vector free period, and to avoid imports of pregnant animals. The surveillance for BT in wild cervids will be continued during next hunting season.

Reference:

Shaw AE, Monaghan P. et al.(2007)Development and initial evaluation of a real-time RT-PCR assay to detect bluetongue virus genome segment 1. Journal of Virological Methods, 145;115-126.

# ORAL: IMMUNIZATION WITH A RECOMBINANT BACULOVIRUS ENCODING A FUSION OF THREE AFRICAN SWINE FEVER VIRUS (ASFV) ANTIGENS UNDER THE CONTROL OF THE MAMMALIAN CMV PROMOTER PROTECT PIGS AGAINST A SUBLETHAL CHALLENGE WITH ASFV

KEIL, GÜNTHER M.[1]; ARGILAGUET, JORDI M.[2]; PÉREZ-MARTÍN, EVA[2]; ESCRIBANO, JOSE M.[3]; RODRÍGUEZ, FERNANDO[2]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]; CENTRE DE RECERCA EN SANITAT ANIMAL[2]; INSTITUTO NACIONAL DE INVESTIGACIÓN Y TECNOLOGÍA AGRARIA Y ALIMENTARIA BIOTECNOLOGÍA[3]

Key words: ASFV, BacMam, vaccine

Previous studies in our lab have shown that fusion of the soluble fraction of the African swine fever virus (ASFV) haemmaglutinin (sHA) to two immunodominat ASFV antigens (PQ) enhanced both the humoral and cellular responses induced in pigs upon DNA vaccination. Unfortunately, no protection against a lethal challenge with ASFV was afforded.

In order to enhance the immune responses induced against these antigens, we decided to use a novel strategy for immunization which is based on the in vivo inoculation of recombinant baculovirus carrying mammalian cell-active expression cassettes (BacMam technology).

Recombinant baculoviruses encoding sHAPQ under the control of the mammalian CMV promoter (same mammalian promotor used in the DNA vaccination protocols) were isolated. The resulting BacMam, BacCMV-sHAPQ, expressed very efficiently the ASFV antigens in mammalian cells upon in vitro transduction. Initial immunization experiments demonstrated that two of three pigs vaccinated with BacMAM-sHAPQ were fully protected from viremia after in vivo challenge with a sub-lethal dose of ASFV.

While protection against γviremia perfectly matched with the presence of a large number of specific IFN secreting T-cells in the blood of the animals at day 10 after challenge, the presence of high T-cell precursors before challenge seemed to correlate with total protection from disease symptoms such as fever.

Further experiments will be performed in the future aiming to study the potential of these vaccines to confer protection against a much more aggressive challenge with a lethal dose of ASFV

# ORAL: EFFICACY EVALUATION OF GOATPOX VIRUS VECTORED PESTE-DES-PETITS-RUMINANTS VACCINE IN FIELD

BU, ZHIGAO[1]; CHEN, WEIYE[1]

HARBIN VETERINARY RESEARCH INSTITUTE (HVRI)[1]

Key words: goatpox virus, PPR, vectored vaccine, field trials

In 2007, the emerging of the peste-des-petits-ruminants virus (PPRV) in southwest board area posed pandemic threaten to China, and a DIVA vaccine is urgently need to be developed for potential pandemic. Here, we constructed a recombinant attenuated goatpox vaccine strain expressing PPRV H protein gene by a quick and efficient selection-purification method, and the safety and immunogenicity of this recombinant vaccine was confirmed in laboratory tests. After that, extensive field trials with different doses and by different inoculation routines in goats and sheep were conducted to evaluate the possibility of practical uses of this candidate vaccine in future. The results showed that the minimum dose of 10000PFU and two inoculation apart of 3~4 weeks are necessary to ensure a 90% higher positive conversion of serum neutralization antibodies against goatpox (titer≥20) and PPR (titer≥10), and also necessary to ensure 6 months longer duration of serum neutralization antibodies positive to goatpox and PPR, especially for goats and sheep pre-immunized with goatpox attenuated vaccine. There is no significant different in serum neutralization antibody conversion rates and titers between intramuscular and intradermal inoculations, goats and sheep species, 3 months ago and 6 or 12 moths ago pre-immunized with attenuated goatpox vaccine. The boost vaccination with single inoculation showed significant re-boost neutralization antibody responses to goatpox and PPR in goats and sheep that were primed 6 months ago. These results suggest that our goatpox virus vectored vaccine is practical DIVA vaccine candidate for the control of PPR and goatpox in future. The quality of vaccination in field animals could be monitored by detections of PPR and goatpox specific serum neutralization antibodies.

# ORAL: CAN VACCINATION PREVENT TRANSPLACENTAL TRANSMISSION OF BTV-8 ?

GALLEAU, STEPHANIE[1]; HAMERS, CLAUDE[1]; BLOSSE, AMANDINE[1]; BOLON, ARNAUD[2]; BLANCHET, MICHEL[1]; GOUTEBROZE, SYLVAIN[1]

MERIAL S.A.S., CENTRE DE RECHERCHE DE SAINT-VULBAS[1]; C/O MERIAL[2]

Key words: Bluetongue virus, cattle, transplacental transmission, vaccination, RT-PCR

INTRODUCTION

Bluetongue virus (BTV) causes an infectious, non-contagious, Office International Epizooties (OIE) listed disease of wild and domestic ruminants. It is transmitted between ruminant hosts through the bites of certain species of Culicoides midges. Recent BTV serotype 8 (BTV-8) outbreaks in Northern Europe have seriously affected the cattle and sheep populations. During these outbreaks, in-utero contaminations of calves have been evidenced. Little is known concerning the efficiency of this mechanism and on its frequency. It is however seen as a way for the virus to spread in the absence of vector midges, allowing overwintering.

While there are indications that some of the licensed BTV-8 vaccines are able to prevent BTV-8 contamination following insect bites, this newly described mode of contamination raises the question of the ability of vaccines to protect from transplacental contamination.

We report a field experience of a natural BTV-8 contamination in a herd that occurred in 2008 in France in the course of a controlled field safety study where pregnant cows were either vaccinated with an inactivated experimental bivalent vaccine containing BTV-8 or remained unvaccinated (control animals). The approximate date of contamination and the frequency of contamination (RT-PCR) of the vaccinated and control cows and of their newborn offspring were determined. The results allowed to estimate a rate of possible vertical contamination and to observe the protection afforded by immunization of the dams against the transplacental transmission.

MATERIAL AND METHODS

Ninety-six pregnant cows, at various stages of gestation and belonging to a BTV-free dairy farm were randomly allocated to 2 groups of 48 animals, on the basis of their pregnancy status. One group was subcutaneously vaccinated twice (day 0 and 28) with 1 mL of an inactivated experimental bivalent vaccine containing BTV-8 (Merial). The other group was left unvaccinated and served as control.

The BTV-8 epizooty that occurred in France in 2008 spread into the area of the study after its beginning. Five months after first vaccination, a natural BTV-8 contamination of the herd was confirmed following a clinical suspicion of contamination in a control cow. All cows that had not yet delivered or that had delivered very recently (within the last 4 days) were tested for BTV serology (ELISA) and viraemia (RT-PCR). All their respective newborn calves were tested for viraemia (RT-PCR) in their first days of life.

RESULTS

Cow contamination: All cows were sero-negative at inclusion in the study. Following the suspicion of contamination, serological analysis revealed that not only almost all vaccinates were sero-positive (as expected) but that almost all controls had also sero-converted, thus confirming the contamination. Furthermore, almost all controls were RT-PCR positive at the date of sampling whereas all vaccinates were RT-PCR negative (as expected as well).

Transplacental contamination: Among the 24 calves born from the control cows and tested for viraemia within 4 days after birth, 10 were RT-PCR positive which suggest transplacental contamination. None of the 21 calves born from the vaccinated cows and tested for viraemia within the same period of time after birth was RT-PCR positive.

CONCLUSION

These results show that transplacental transmission of BTV-8 is a rather efficient process as more than 40% of the contaminated cows gave birth to RT-PCR positive calves. In contrast, immunization of the pregnant dams with the tested vaccine appears to have completely prevented transplacental contamination of the calves.

# ORAL: VETERINARY RISK OF DEER IN ROBUST NATURAL CORRIDORS IN THE NETHERLANDS

DE VOS, CLAZIEN[1]; GROOT BRUINDERINK, GEERT[2]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]; ALTERRA, WAGENINGEN UR[2]

Key words: disease transmission, qualitative risk assessment, fallow deer, red deer, roe deer

In the Netherlands robust natural corridors will be created to connect natural areas and to enlarge the habitat of plant and animal species. Some of these corridors will allow for migration of wild ungulates. Extension of the range of red deer (Cervus elaphus) and fallow deer (Dama dama) might, however, facilitate spread of animal diseases, both within these populations and to domesticated animals. Roe deer (Capreolus capreolus), on the contrary, already present a potential veterinary risk for the livestock sector since they live throughout the country. The objective of this study was to assess the additional risk posed by the future presence of red deer and fallow deer in robust nature links.

For this purpose a qualitative risk assessment was conducted taking into account
(a) expected number of deer in future robust natural corridors;
(b) prevalence of specific diseases in deer populations in the Netherlands;
(c) differences in susceptibility to and excretion of pathogens in wild ungulates;
(d) disease transmission routes from wild deer to livestock.

Diseases taken into consideration were foot-and-mouth disease (FMD), bluetongue (BT), infectious bovine rhinotracheitis (IBR), bovine virus diarrhoea (BVD), paratuberculosis, Q-fever, and babesiosis.

Main results of the risk assessment are:
• Overall deer densities will increase by approximately 50%.
• There is no structural monitoring of diseases among wild deer in the Netherlands. Up till present, only the presence of BT, paratuberculosis, and possibly IBR has been confirmed in red deer. No diseases have been observed in fallow deer and roe deer.
• Red deer and fallow deer are members of the subfamily Cervinae, whereas roe deer are member of the subfamily Odocoilinae. The latter family seems to be more susceptible to disease (e.g. FMD). However, information on differences in susceptibility and excretion is scarce.
• Red deer and fallow deer live in larger groups than roe deer increasing the probability of intraspecific transmission by direct contact (FMD, IBR, BVD, Q-fever).
• Red deer and fallow deer have a larger niche overlap with cattle than roe deer increasing the probability of interspecific transmission via indirect contact (paratuberculosis) and vectors (BT, babesiosis).

Taking all this into account, we concluded that migration of red deer and fallow deer in robust natural corridors will result in a slight increase of the general veterinary risk for the Dutch livestock sector. Quantification of the risk was not possible because no disease prevalence data are available for red deer, fallow deer, and roe deer in the Netherlands. Given the lack of historical evidence of disease transmission from roe deer to livestock, we assume that the present veterinary risk is small. Furthermore, we expect that the veterinary risk will remain small, despite a slight increase due to the presence of red deer and fallow deer.

# ORAL: TOGGENBURG ORBIVIRUS (TOV): GENOMIC FEATURES, EXPERIMENTAL INFECTION, PATHOLOGY AND EPIDEMIOLOGY OF A NOVEL BLUETONGUE VIRUS DETECTED IN GOATS FROM SWITZERLAND

THUER, BARBARA[1]; CHAIGNAT, VALERIE[1]; WORWA, GABRIELLA [1]; HOFMANN, MARTIN[1]

INSTITUTE OF VIROLOGY AND IMMUNOPROPHYLAXIS[1]

Key words: Toggenburg orbivirus (TOV), Bluetongue, Serotype 25, Switzerland, Goats

A novel bluetongue virus (BTV) termed "Toggenburg orbivirus" (TOV) was detected in 2 Swiss goat flocks. Clinically healthy goats were positive for BT antibodies in ELISA and real-time RT-PCR. However, the amplification curves showed deltaRn values much lower than usually observed with BTV. Confirmatory rRT-PCRs yielded negative results. None of four strongly ELISA-positive sera showed neutralizing activity with any of the 24 BT reference serotypes (1).

The viral genome was completely sequenced. BLAST analysis and dendrogram construction revealed that the TOV is closely related to viruses from the BT serogroup although some of the genome segments are quite distinct from the known 24 BTV serotypes. However, since the gene encoding VP2, which determines the serotype of BTV, was clearly placed among the 24 known BTV serotypes, we propose that TOV represents a so far unknown 25th serotype of BTV (2).

In the field, no clinical signs were observed in TOV-infected adult goats; however, several stillborn and weak born kids were reported. Experimental infection of goats and sheep using TOV-positive blood from field cases was performed to assess the pathogenicity of TOV. Goats did not show any clinical or pathological signs, whereas in sheep mild BT-like clinical signs were found. Necropsy of sheep demonstrated BT-typical haemorrhages in the wall of the pulmonary artery. Viral RNA was detected in organs of experimentally infected animals.

Furthermore, a serosurveillance study in goats using sera collected in early 2008 was conducted. Preliminary data show that in Ticino, a so far BTV free region south of the Alps, approximately 70% of tested goats reacted positive in the BT ELISA. Positive animals were distributed randomly within the surveyed region and TOV virus could be detected and typed by partial segment 2 sequencing in samples collected in autumn 2008. In the Grison, a region in the eastern part of Switzerland, 18% of goat sera were BT ELISA-positive, and in one flock TOV was detected. A TOV-positive, clinically ill goat was found in the central part of Switzerland in late 2008. These data indicate that TOV is circulating in Switzerland.

In a few TOV-positive goat herds cattle, alpacas or sheep were also tested. They were negative for BT antibodies and TOV. Testing of more cattle and sheep samples from the regions with high TOV incidence in goats is ongoing, and results will be presented.

So far, it has not been possible to propagate the virus outside of its ruminant host, neither in any mammalian or insect cell line nor in embryonated chicken eggs.

Up to now, the epidemiology of TOV infection is unclear, since most of the goats were infected while being kept on alpine pastures higher than 1500 m above sea level. Because goats are infrequently tested for BTV in Switzerland, it is possible that TOV has been circulating unrecognized already for a long time in the goat population. Numerous additional open questions remain on the molecular evolution of TOV, its pathogenesis, host and organ tropism, phenotypic characterization and growth characteristics. However, before these questions can be addressed, the inability of TOV to replicate in vitro remains the major bottleneck in TOV research.

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# ORAL: INDICATORS TO MONITOR POTENTIAL EFFECTS OF CLIMATIC CHANGE ON ANIMAL HEALTH: ESTABLISHING PRIORITIES

BOONE, IDES[1]; BRITS, ETHEL[2]; VERHAGEN, BART[3]; VAN NIEUWENHUYSE, AN[2]; DISPAS, MARC[1]; VAN DER STEDE, YVES[1]; VAN OYEN, HERMAN[1]; NAJI, AZIZ[4]

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE (VAR)[1]; SCIENTIFIC INSTITUTE OF PUBLIC HEALTH[2]; FPS PUBLIC HEALTH, FOOD CHAIN SAFETY & ENVIRONMENT[3]; FPS SCIENCE POLICY[4]

Key words: animal health, climate change, indicator, monitoring

Nowadays, there is consensus among scientists that climate change is unequivocal and very likely to be caused by human activities. In order to take effective measures of mitigation and adaptation, an inventory of potential effects caused by climate change on the human and animal health has been carried out for Belgium. In the present study the potential effects of climate change on animal health, which are diverse and often surrounded by many uncertainties, are highlighted. Direct effects include heath-related effects such as those related to ozone depletion (skin, eye lesions,...) and the health effects to due extreme weather events (storms, floods,...). Indirect effects are mainly related to vector-borne, water-borne, food-borne and other infectious diseases. In addition to climate change, environmental changes (e.g. changes in land use and land cover) as well as socio-economic factors (e.g. increased trade and tourism) can also affect animal health.

Based on (inter)national literature review (OIE, 2008) and expert consultations, a list of biotic and abiotic indicators, useful for monitoring and relevant for Belgium, was established by means of a matrix. In particular, indicators related to vector-borne diseases will be highlighted as they are considered to pose the highest threat. Following criteria are taking into account in the prioritization of indicators to monitor animal health effects due to climate change: policy relevance, expected risk magnitude, the uncertainty of the relationship between climate change and health effect, and the mitigation and adaptation possibilities to cope with the health effects. In addition, the availability and the quality of (inter)national information sources and existing surveillance plans are considered during the prioritization. Whenever possible, indicators are sought to be comparable among EU Member States but also other (third) countries.

The results obtained here is the first step in order to set up an integrated long-term monitoring tool to evaluate the effects of climate change on both animal and human health in Belgium. This tool is intended to be dynamic and interactive and provide a platform for scientists, stakeholders and decision-makers in order to help policy-makers taking appropriate measures towards climate change mitigation and adaptation.

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# ORAL: BLUETONGUE DISEASE – EVALUATION OF THE MONITORING AND SURVEILLANCE SYSTEM OF THE EUROPEAN UNION

FROEHLICH, ANDREAS[1]; GETHMANN, JOERN[1]; STAUBACH, CHRISTOPH[1]; CONRATHS, FRANZ J.[1]; METTENLEITER, THOMAS C.[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: Bluetongue, Monitoring, Surveillance, EC regulation

In August 2006 bluetongue disease, a non-contagious disease of ruminants was detected in The Netherlands, Belgium and Germany. Until the end of the year more than 2,000 cases were reported and five countries were affected. In October 2006 a working document was developed by DG-SANCO to provide uniform guidelines for monitoring and surveillance systems for bluetongue disease in the European Union. This working document was the basis for "Commission Regulation (EC) No 1266/2007 on implementing rules for Council Directive 2000/75/EC as regards the control, monitoring, surveillance and restrictions on movements of certain animals of susceptible species in relation to bluetongue of 26 October 2007".
Until the end of 2008 the regulation was modified several times. The aims of the monitoring and surveillance systems in the regulation are to detect the introduction of new bluetongue serotypes and to demonstrate the absence of certain bluetongue serotypes. Other objectives may include the demonstration of the absence of bluetongue virus circulation.
Furthermore it is the basis for exempting certain animals of susceptible species from the exit ban.

The goal of our study is to verify whether the monitoring and surveillance systems are appropriate to ensure the aims of the EC regulation. Particularly we examined the sentinel program regarding the early detection of bluetongue cases. Therefore we analysed the epidemiologic data of BTV-8 collected in Belgium, France, Germany, Luxembourg and The Netherlands in 2006 and calculated the monthly incidence. Furthermore we included data from the monitoring and surveillance program in 2007 in Germany and corrected the power of the sentinel program. Subsequently we compared these data with the specifications mentioned in the regulation.

# ORAL: NEW MOLECULAR APPROACHES FOR DETECTION OF AFRICAN SWINE FEVER VIRUS

FERNANDEZ-PINERO, JOVITA[1]; STAHL, KARL[2]; HERTJNER, BERNT[3]; GALLARDO, CARMINA[1]; HAKHVERDYAN, MIKHAYIL[2]; RONISH, BONNIE[4]; WANGH, LAWRENCE[4]; ALLAN, GORDON[3]; ARIAS, MARISA[1]; BELAK, SANDOR[2]

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[1]; NATIONAL VETERINARY INSTITUTE (SVA)[2]; QUEEN'S UNIVERSITY OF BELFAST[3]; BRANDEIS UNIVERSITY[4]

Key words: ASFV, molecular diagnosis, UPL, LATE-PCR, LAMP

African Swine Fever (ASF) is a highly contagious infectious disease affecting Suidae, caused by a DNA virus of the Asfarviridae. It is a notifiable disease, endemic in large parts of Sub-Saharan Africa and in Sardinia, producing great economic losses. In 2007, ASF emerged in the Caucasus for the first time, and has since spread to several countries in the region. Molecular diagnosis of ASF relies on a limited number of PCR methods (1,2,4,6). Within the recently started EU project ASFRISK, new tools are being developed using novel technologies, to update and improve current molecular techniques for detection of ASF virus (ASFV), and to be used under different laboratory and field conditions, mainly in countries affected by ASF.

Universal Probe Library (UPL), recently commercialized by Roche Applied Science, is a collection of short hydrolysis LNA probes, originally designed for gene expression analysis and offered as a universal detection system. Currently, UPL probes are applied also for pathogen detection, main advantages being reasonably low cost, short time of delivery, and ready-to-use format. The combination of a specific primer set and an appropriate UPL probe will allow specific and sensitive detection of ASFV by real-time PCR at a comparably lower cost.

LATE (Linear-After-The-Exponential)-PCR is an advanced asymmetric PCR that provides several advantages, such as increased multiplexing capacity and faster thermocycling, compared to currently used PCR chemistries (5). The LATE technology is exclusively licensed by Smiths Detection, and the developed ASF assay will be adapted to their portable PCR platform BioSeeq to provide a robust, powerful and simple-to-use diagnostic system for onsite detection of ASFV in a wide range of environmental conditions.

Loop-mediated isothermal amplification (LAMP) is based on amplification of nucleic acids without the need of PCR equipment (3). The comparative simplicity of the technology makes LAMP adaptable to front-line testing in regional laboratories, simple diagnostic situations and even to pen-side testing. Lamp technology also has the potential of becoming a method for rapid first-line diagnosis of ASF.

The design of real-time PCR assays using UPL probes, LATE and LAMP technology has been focused on highly conserved regions of the genome. A collection of ASFV isolates has been tested on this panel of assays designed within the VP72 and 9GL coding genes, for a preliminary evaluation of the detection competence, giving promising results.

This work presents the design and the first results of new nucleic acid-based assays for the improved detection of ASFV, using novel molecular approaches. The optimised assays will improve the robustness of the ASF diagnostic arsenal and will offer additional diagnostic tools, suitable for use in well-equipped international and national reference laboratories, in basic regional and local laboratories, and for rapid first-line diagnosis at the pen-side.

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# ORAL: DETECTION OF FMD INFECTION IN VACCINATED ANIMALS (DIVA) IN TURKEY: COMPARISON OF TESTS TO DETECT FMDV CARRIER ANIMALS

COKCALISKAN, CAN [1]; GRAINGER, PHILIPPA [2]; ALKAN, MUSA [1]; PATON, DAVID[2]; EBERT, KATJA[2]; OZYORUK, FUAT[1]; PARIDA, SATYA[2]

FOOT AND MOUTH DISEASE INSTITUTE (SAP)[1]; INSTITUTE FOR ANIMAL HEALTH (IAH)[2]

Key words: FMD, serosurveillance, carrier, DIVA test

Detection of FMD Infection in Vaccinated Animals (DIVA) in Turkey: Comparison of tests to detect FMDV carrier animals

Can Cokcaliskan1&2, Philippa Grainger1, Musa Alkan2, David Paton1, Katja Ebert1, Fuat Ozyoruk2 and Satya Parida1*

1 Institute for Animal Health, Pirbright, UK
2 SAP FMD Institute, Ankara, Turkey
* Corresponding author; satya.parida@bbsrc.ac.uk

Within Europe, there is a growing desire to make greater use of vaccination for the control of future outbreaks of foot-and-mouth disease (FMD) and to slaughter fewer livestock. Animals immunised with FMD vaccines can be protected against clinical signs of FMD and shed lesser amounts of virus following challenge infection. However, susceptible animals exposed to viral challenge after vaccination can still be sub-clinically infected, and in the case of ruminants this can lead to persistent oro-pharyngeal infection with a duration of months or even years. The latest recommendations from the OIE are that countries that use FMD vaccination for the control of the disease can only attain the trade status of "FMD-free" once they have conducted serosurveillance to show absence of infection. Following use of vaccination, serosurveillance can only be carried out effectively using tests that detect infection in vaccinated animals (DIVA). To evaluate the efficiency of detection of FMDV carrier animals in field situation a collaborative study has been undertaken between SAP, FMD Institute, Ankara and Institute for Animal Health, Pirbright. 250 serum, saliva and oro-pharyngeal fluids had been collected from cattle from vaccinated farm in Turkey after an outbreak of A Iran, 2005 FMDV. The samples are being analysed in real-time RT-PCR, virus isolation, different NS antibody tests, salivary IgA test and IgM test to detect the FMDV carrier animals and to find out the efficiency of individual test in detecting persistent infection. Till date 160 serum samples were scored positive in Cedi NS test and 75 oro-pharyngeal saples were detected positive in reali-time RT-PCR. The analysis of final results and the use of various tests for detecting FMD carrier will be discussed during presentation.
Acknowledgement:This work was supported by the EU Network of Excellence, EPIZONE (Contract No Food-CT-2006-016236) and Defra, UK, Grant No SE1125. Can Cokcaliskan is a visiting scientist at Pirbright, UK and supported by EPIZONE short term EPIZONE mission. DP is a Jenner fellow and SP is adjunct professor to Murdoch, University, Australia.

# ORAL: EVIDENCE OF RECENT RIFT VALLEY FEVER VIRUS CIRCULATION IN MAYOTTE, A FRENCH ISLAND OF THE INDIAN OCEAN

CETRE-SOSSAH, CATHERINE[1]; BILLECOCQ, AGNÈS[2]; DEFERNEZ, CÉDRIC[3]; FAVRE, JACQUES[3]; BOULOY, MICHÈLE[2]; MARTINEZ, DOMINIQUE[1]; ALBINA, EMMANUEL[1]

CENTRE DE COOPÉRATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT (CIRAD)[1]; INSTITUT PASTEUR[2]; SERVICES VÉTÉRINAIRES DE MAYOTTE[3]

Key words: Rift valley fever ELISA Mayotte

Rift Valley Fever (RVF) is a serious emerging arthropod-borne viral anthropozoonosis caused by a Phlebovirus named Rift Valley Fever Virus (RVFV) belonging to the Bunyaviridae family. It is reported primarily to infect sheep, cattle and goats, producing high mortality in newborn animals and abortion in pregnant animals. It is also a zoonosis causing epidemics of flu-like syndrome with some cases of haemorrhagic or neurologic complications in human population across Africa. Outbreaks of the disease occur when particularly heavy rains favour the breeding of the mosquito vectors (Swanepoel and Coetzer 2004). Many sub-Saharan tropical and sub-tropical countries in Africa have reported outbreaks of RVF and the disease is encountered in an enzootic or epizootic form along the east and south coast of Africa and also in Madagascar. Periodic large-scale epidemics occurred in African countries like Mauritania and northern Senegal in 1987 and 1998 (Digoutte 1989, Meegan 1989, Ksiazek 1989), Madagascar in 1990-1991 (Morvan 1992), as well as Kenya, Somalia and Tanzania in 1997-1998 (Woods 2002). The last epidemics of RVF was in Comoros (1 child infection), and Madagascar which has already reported 17 human deaths and 418 suspected infections in Mid-April 2008 (Promed 2007, 2008). In this context, the veterinary services of Mayotte Island, which is 67 km from the most southern island of Comoros, decided to set up three different serological studies. The analysis (detection of IgG antibodies to RVFV) was performed with a competitive ELISA recently validated in different species such as humans, domestic and wild ruminants (Paweska 2005). A first serological survey was focused on samples collected in 2007 in one area selected for a risk of illegal introduction of animals from Comoros. 79 cattle and 23 illegally imported goats located in eighteen different herds were sampled. 13 samples were found positive for IgG and 3 for IgM. Only one illegally imported goat scored positive and only for IgG. A follow-up of the negative bovines in April 2008 and of additional goats from illegal import led to the conclusion of a recent circulation of the virus since 2 goats over 29 were found IgM positive and one bovine seroconverted between November 2007 and March 2008. Therefore, it was decided to test a total number of 304 bovine sera collected between June 2007 and May 2008 distributed all over the Island. An overall percentage of 11% (95%IC:7-14) was detected in the whole island. Finally, in order to set up a serosurveillance of this disease, 5 herds with a range of 4 to 35 animals in each herd are followed up every 6 to 8 weeks. So far, none of the animals seroconverted. These studies illustrate the recent circulation of RVF in Mayotte. Studies are ongoing to detect a possible reactivation of the virus circulation and also to trace back the possible period of initial introduction of the virus in the island.

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# ORAL: DEMONSTRATION OF EFFICACY OF DUVAXYN WNV UNDER FIELD CONDITIONS

CHIANG, YU-WEI [1]; HATHAWAY, DIANE[1]; JENNINGS, NEIL[1]; NG, TERRY[1]; CHU, HSIEN-JUE[1]

FORT DODGE ANIMAL HEALTH[1]

Key words: West Nile Virus, Vaccine, Efficacy

A field safety and efficacy study of Duvaxyn WNV was conducted. This study was conducted at three sites in the United States where West Nile virus (WNV) was endemic during the mosquito season of 2005. At the end of the field study, the horses located at all three sites were assessed for natural exposure by ELISA for specific IgG and IgM responses. Positive IgM antibody response was detected in 7 of 15 control horses at the California site and 2 of 15 control horses at the Iowa site. The horses at these two sites had most likely been exposed to natural WNV infection. However, no IgM response was detected in any of the vaccinated horses at the same locations. The control horses which were housed communally with the vaccinates at the New Jersey site were found to remain negative to WNV, indicating absence of natural exposure to the virus. This group of horses was then transported to Fort Dodge Animal Health and experimentally challenged with live WNV. After challenge, viremia was detected in 5 out of 15 control horses while none of 13 vaccinates was detected with viremia. The vaccine efficacy is 100% (95% CI 12.4, 100). Results of this study clearly indicate that Duvaxyn WNV, administered twice 3 to 4 weeks apart to horses under field conditions, provides protection against WNV infection.

# ORAL: WILD BIRD MOVEMENTS AS A WAY OF INTRODUCTION OF TRANSBOUNDARY EMERGING DISEASES: THE CASE OF CRIMEAN CONGO HAMORRHAGIC FEVER

MARTINEZ, MARTA[1]; DE LA TORRE, ANA[1]; IGLESIAS, IRENE[1]; BOSCH, JAIME[1]; MUÑOZ, MARIA JESUS[1]

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[1]

Key words: birds, ticks, vectors, transboundary, risk

The migratory movements of wild birds are a recognized way of entrance of many current transboundary diseases, such as avian influenza, West Nile fever or Crimean Congo haemorrhagic fever (CCHF). In the latter, ground-feeding birds can act as mechanical carriers of the immature stages of vector ticks that can be infected with CCHF virus, like Hyalomma marginatum and Ixodes ricinus. CCHF is endemic in many countries in Africa, Europe and Asia, and during the past years outbreaks have been recorded in Turkey, Kosovo, Albania, Iran, Pakistan, Mauritania and South Africa. Although widespread, Hyalomma and Ixodes are limited by opposite bioclimatic factors. Hyalomma ticks are generally densely distributed in Africa and in Mediterranean ecosystems while Ixodes ricinus has a more Northern distribution corresponding to a continental ecosystem.

Spain is a meridional country with a high ornithological relevance in the migratory movement between Eurasia and Africa. Many ground-feeding birds migrate to Spain from West and North Africa each Spring for the breeding season. Currently, most of the immature stages of the Hyalomma ticks that could be arriving in Spain with the birds from Africa would not survive the climatic conditions on arrival. However, warming due to climate change may increase the current survival and distribution of ticks together with the possible spread of CCHF. In this study we present a methodology to assess the risk of introduction of CCHF from Africa based on climate change parameters that can influence the biology of vectors that are carried by migratory birds, using Spain as an example. The 2 main sources analyzed are: a) the Spanish database of migratory birds' ringing records, that has been used to identify the ground-feeding wild species that connect African countries to different locations in Spain; and b) the availability of climatic information, mainly monthly temperatures and relative humidity, from more than 2500 climate stations of the Spanish Estatal Climatic Agency (AEMET) that have been modified accordingly in time and space in relation to the results of the recent European studies on climate change. Results show the distribution of possible risk factors that can contribute to the introduction of CCHF in a currently free country.

In conclusion, this study shows an approach for the assessment of the risk of CCHF introduction by migratory wild birds, a true risk for Spain as a consequence of climate change. The same approach could be applied to the rest of European countries should the necessary data be available. Improvement of the knowledge about avian migration patterns, the agents that bird ticks harbour and the alterations in the ticks' habitats associated to climate change might be useful in helping to predict future outbreaks of infection due to emerging zoonotic tick-borne pathogens.

# ORAL: AN EXPERIMENTAL INFECTION OF EUROPEAN BREED SHEEP WITH RIFT VALLEY FEVER VIRUS

ABAD, FRANCESC X. [1]; BUSQUETS, NÚRIA[1]; RODRÍGUEZ, FERNANDO[1]; SOLANES, DAVID[1]; DOMINGO, MARIANO[1]; BRUN, ALEJANDRO[2]

CENTRE DE RECERCA EN SANITAT ANIMAL[1]; CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[2]

Key words: Rift Valley fever; pathogenesis; experimental infection;

Rift Valley fever (RVF) is a zoonotic disease showing episodic outbreaks in Sub Saharan countries, Egypt and the Arabian Peninsula eventually associated to climatic conditions. The disease is caused by an arbovirus competent in many mosquito species present in most European countries. Many European regions have optimal habitats in which the virus could persist in the event of an unwanted introduction of the virus within European boundaries. Increasing our understanding of the disease is therefore highly desirable to increase our preparedness to fight this important disease. In this study we tried to establish a reproducible infection model of RVF in a European sheep breed after the inoculation of four different South African isolates of the virus. Groups of 4 sheep of 6-8 weeks old were inoculated subcutaneously with 105 TCID50 of each isolate. Viremia titers were estimated by real time PCR and presence of viable virus confirmed by virus isolation. Different tissues were analysed for specific pathological changes by histopathology and/or immunhistochemistry. Leukocyte populations were also studied looking for immune correlates of infection. Shedding of RVFV and horizontal transmission to non-infected, in contact lambs was also investigated. The results will be discussed.

# ORAL: BLUETONGUE IN THE NETHERLANDS: PREPAREDNESS ON THE UNEXPECTED

VAN RIJN, PIET A.[1]; VAN GENNIP, RENÉ[1]; GEURTS, YVON[1]; VELDMAN, DANIEL[1]; BOONSTRA, JAN[1]; KRAMPS, HANS[1]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]

Key words: Diagnostics Bluetongue serotypes

Several serotypes of Bluetongue virus (BTV) were expanding northwards in 2008, in particular BTV1. After introduction of the unexpected BTV8 in 2006, the national reference institute was keen to be prepared on incursions of European, but also of other serotypes of BTV. For this preparedness, we started to develop serotype-specific real time RT-PCR assays, but for several serotypes. Follow-up research of all panBTV-PCR-positives was performed in 2008. First, sequencing of the amplicon of our panBTV-PCR assay was performed. In August, panBTV-PCR-positives were identified as or very closely related to BTV8\2006. Up to three nucleotide changes were found in the variable amplicon of the diagnostic rRT-PCR assay based on segment 10. These were considered as "normal" genetic drift. The serotype 8 was confirmed by a serotype-specific rRT-PCR assay. Starting from early September, three clinically diseased (particularly coronitis) cattle were found in BTV8-vaccinated and unvaccinated herds with 11 nucleotide differences in the respective amplicon of segment 10. PanBTV-PCR-positives were not detected by serotype-specific rRT-PCR assays for BTV-8. Conventional serotype-specific RT-PCRs based on segment 2 for European BTV serotypes were also negative, although homology based the amplicon suggested a genetic relationship with BTV2 isolates. Extensive serotyping by Institute of Animal Health with primersets for all 24 serotypes identified the isolate as BTV serotype 6. Sequencing showed that most segments of BTV6\net2008 are genetically closely related to that of the BTV6 reference strain, except for segment 10. This segment matched closer to that of representatives of serotype 2. Segment 2, the major representative of the serotype, differs on five positions from that of the reference BTV6 strain. Comparison with segment 2 of MLV-6 from South Africa (kindly provided by Onderstepoort Veterinary Institute) showed one, silent nucleotide change. Sequencing and comparisons of other segments are in progress.
In early November, it became clear that a bull positive for BTV-1 has been imported in the Netherlands at October 8th, 2008. The entire herd and the 1 km zone was completely sampled for BTV screening to evaluate the spread of BTV-1. Four additional panBTV-PCR-positive animals were found. Three animals originated from France and were BTV8 positive. The fifth animal was BTV6 positive, and could be traced to the original area of the other BTV6 positive animals in the Netherlands.

# ORAL: BLUETONGUE VIRUS SEROTYPE 6 IN THE NETHERLANDS

VAN RIJN, PIET A.[1]; GEURTS, YVON[1]; VAN GENNIP, RENÉ[1]; VELDMAN, DANIEL[1]; BOONSTRA, JAN[1]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]

Key words: emerging epidemiology bluetongue transmission

In September, 2008, three clinically diseased (particularly coronitis) cattle were found positive for BTV6. These animals were vaccinated (2) or not vaccinated (1) for BTV8. The latter was are very small holding and positive for BTV8 and BTV6. The involved farms and the respective 1 km zones were screened to evaluate the spread of BTV6. Animals of the infected holdings were tested by panBTV-PCR and by serum neutralization assay (SNT). No other PCR-positive animals were found on these farms. SNT results confirmed BTV8 vaccination or BTV-8 circulation in previous years. Only one additional seropositive animal for serotype 6 was found. Neutralization titers to other serotypes than 6 and 8 were not found on these farms. Animals in the 1 km zones were tested by panBTV-PCR. Only a very few additional BTV6-positive animals were found. No BTV8-positive animals were found. Further, no new clinical diseased animals were found. Since then, several BTV6-positive animals were found accidently by testing for export purposes. In all cases, no additional positives were found on the farm. Resampling showed that most PCR-positives remained positive for at least one month. To evaluate the prevalence of BTV6 prior the first findings in September, sera of all seropositive clinical suspicions of 2008 were tested for neutralizing antibodies against BTV6. No neutralizing titers against BTV6 were found. This indicates that BTV6 was not in the Netherlands before September 2008.

One BTV6-positive animal was moved to CVI and served as BTV6 source for an animal trial. This animal remained panBTV-PCR-positive for > 80 days after the first sampling, and contained neutralizing titers against BTV8 (by vaccination and/or natural infection) and against BTV6 (by infection). Sheep and cattle, seronegative for BTV, were successfully infected with BTV6\net2008. These groups of animals showed no clinical signs typical for BT. Animals became PCR-positive at four days post infection and seroconverted as could be expected. All animals remained PCR-positive for at least 25 days post infection, the end of the experiment. We conclude that BTV6\net2008 is not virulent for sheep and cattle, however both species were infected by BTV6\net2008 and resulted in a viraemia (by PCR) of at least 25 days and seroconversion. Height and length of the viraemia as measured by infectious BTV were not measured. Field data suggests an extreme low transmission of BTV6\net2008. BTV6-positive animals will be resampled to determine the length of the viraemia (by PCR). Implication for the next season will be discussed.

# ORAL: DESIGN OF A ONE-STEP MULTIPLEX RT-PCR FOR THE DETECTION AND THE SUBTYPING OF H1, H3, H5, H7, N1, N2 HUMAN AND AVIAN INFLUENZA VIRUSES

HAMMOUMI, SALIHA[1]; GIL, PATRICIA[1]; ALBINA, EMMANUEL[1]

CENTRE DE COOPÉRATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT (CIRAD)[1]

Key words: influenza, RT-PCR, multiplex, subtype

Influenza is an important viral infection of humans and animals with a high potential for pandemics and a considerable fatality rate. Experts and international bodies agree that rapid detection of influenza virus is vital in combating this major threat to human and animal health. Several conventional or real-time RT-PCR methods for the detection of influenza virus genome have been published. For most of them, primers and probes have been designed on conserved regions based on alignments of few hundreds of sequences from different genes. However, the subtyping of influenza viruses requires the specific amplification of the different hemagglutinin and neuraminidase genes. These two genes are so variable that newly circulating strains may not be detected using primers and probes designed on an insufficient number of strain sequences. Here, we describe the design of three couples of primers and probes for the detection of four different hemagglutinin and two neuraminidase genes of interest for human and avian influenza (H1, H3, H5, H7, N1, N2), based on the alignment of several thousands of sequences available in GenBank. Each couple was designed to detect two subtypes: H1 or H5, H3 or H7 and N1 or N2. The combination of all primers in a one-step multiplex RT-PCR allowed detecting all the expected targets. The amplicons were differentiated by their size on agar gel electrophoresis. In order to discriminate two subtypes detected with the same primers pair, six probes were subsequently designed for the specific detection of individual amplicons using the Taqman® technology. These probes are being validated individually and will be used in the multiplex real-time RT-PCR with the three couples of primers. Due to the limited number of fluorescence filters in real-time PCR machines, the multiplex reaction will be done twice for a single sample during the same experiment using three probes in the first tube and the three others in the second tube. Another solution will be to associate the real-time RT-PCR results using the three couples of primers and the six probes with agar gel electrophoresis. Indeed, a doubt on a subtype due to a fluorescence signal that could correspond to two probes should be eliminated by analysing the size of the amplicon.

In this work, we describe a multiplex RT-PCR allowing the detection and subtyping of the H1, H3, H5, H7, N1 and N2 human and avian influenza viruses. The final objective of this work is to use these primers and probes to develop a rapid one-step multiplex real-time RT-PCR for the 6 subtypes of interest using six fluorescence channels.

# ORAL: DIAGNOSTIC APPROACH TO A WEST NILE VIRUS OUTBREAK IN NORTHERN ITALY ON FALL 2008

MORENO, ANA[1]; LELLI, DAVIDE[1]; BARBIERI, ILARIA[1]; CANELLI, ELENA[1]; TAMBA, MARCO[1]; AVISANI, DOMINGA[1]; BONILAURI, PAOLO[1]; CORDIOLI, PAOLO[1]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL' EMILIA ROMAGNA (IZSLER)[1]

Key words: diagnosis, surveillance, west nile virus, Northern Italy

Introduction

In August 2008 West Nile virus (WNV) reappeared in Italy after about 10 years. The first case was in a horse showing neurological symptoms from the province of Ferrara near the the Po river delta. Serological diagnosis firstly made at the Virology laboratory of IZSLER was then confirmed by the national reference centre of IZS Teramo (CESME). Since then an extensive monitoring program is applied in the high risk area including three regions Lombardia, Emilia Romagna and Veneto. The program is based on: 1) symptomatic surveillance in horses, 2) serological and virological diagnosis in horses stables, 3) control of synantropic domestic and wild birds captured or found dead. Furthermore a vector surveillance plan including capture and identification of mosquitoes and further diagnosis by PCR and virus isolation was applied in infected areas. The results of the WN surveillance carried out in Lombardia and Emilia Romagna regions and the isolation and molecular characterization of some WNV strains isolated in 2008 are here reported.

Material and methods

Samples from brain, heart and kidney of clinically affected horses and of susceptible birds as well as pools of mosquitoes were tested by PCR as described by Scaramozzino et al. (3). Virus isolation was performed on Vero, RK13 and BHK21 cells and the the presence of WNV was confirm by PCR and immuno-peroxidase using Monoclonal antibodies (MAbs). Serum samples for anti-WNV antibody detection were tested by an in-housed MAb-based competitive ELISA.. Sera were also sent to CESME for serologic diagnosis confirmation by neutralisation tests. Total RNA from one isolate and from 1 pool of organs of a horse and 2 pool of mosquitoes was extracted. Viral RNA was reversed transcribed and amplified by using the one step RT-PCR kit (Qiagen, Germany). Nucleic acid sequence analysis was performed on NS5 region on all samples using the primers previously described (3). The partial sequencing of the envelope gene (1) was also carried out on the WNV strain isolated on tissue culture from the organs of a magpie.

Results and discussion

Monitoring program revealed the presence of several outbreaks in horses with a total of 288 serologically positive animals. Clinical sings were observed in 31 horses. Out of these 5 animals died. No isolation was achieved from PCR positive samples of the horse and mosquitoes. Three viral strains were isolated from pool of organs of captured wild birds (two magpies, Pica pica, and one jay, Garrulus glandarius). Partial nucleotide sequence of the genome region coding for the NS5 protein revealed a 100% homology among all the samples originated from Ferrara province i.e. the WNV isolated and RNA obtained from the horse with specific clinical signs and the 2 pools of mosquitoes. In addition a high degree of homology was also observed when this sequence was compared with that of strains isolated in France on 2004 and Spain (2) on 2007. The partial sequence of E gene of the WNV isolate showed a high homology (99,4%) to strains isolated in France in 2000. These results indicate that a new epidemic of WN occurred in Italy caused by a WNV closely related to strains circulating in other European countries. These findings and the fact that the area where the infection took place was close to wildlife nature reserves are consistent with the possible dissemination of WNVs by migrating birds.

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# ORAL: NEW FLUBIRD DATABASE - PLATFORM FOR DATA EXCHANGE AND KNOWLEDGE BUILDING IN AVIAN INFLUENZA SURVEILLANCE

MATHEY, ALEXANDER[1]; STAUBACH, CHRISTOPH[1]; KOWALCZYK, STEFAN[1]; WILKING, HENDRIK[1]; KRANZ, PETRA[1]; RICHTER, SVEN[1]; GLOBIG, ANJA[1]; HARDER, TIMM[1]; CONRATHS, FRANZ J.[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: Avian Influenza Surveillance Database Modelling

To tackle shortcomings in the current understanding of the epidemiology of avian influenza viruses in migratory wild birds, a network of virologists, ornithologists and epidemiologists was established, built on related initiatives and cooperations. Apart from enhancing possibilities of method standardization and sharing of data and expert knowledge, this large-scale cooperation forms the backbone of a targeted surveillance system, with tasks distributed among the partners according to their respective expertise.

As a central instrument for this purpose, a database system was developed to store, manage and analyse data from the different disciplines, as well as additional environmental data. A flexible user management system was implemented, allowing data access rights to be configured independently for different users, and different data types, respectively. Interaction by project participants is possible via a secured internet connection and a web interface, which provides the different tools and modules for data processing.

Emphasis is placed on the integrative process of combining the interdisciplinary data for analysis, which is realized on different levels. Interactive software modules allow for the creation of database queries, targeting parameters shared by the different types of data. The resulting subsets of interest can be ordered, stratified and visualized in form of tables and diagrams, as well as in thematic maps created by means of a linked map server. For example, geo-referenced diagnostic results from sampling can be visualized on maps and overlaid with species-based migration flyway maps that were compiled by the ornithological partners. Established data mining algorithms will be deployed to search the data pool continuously for hidden patterns and trends that could allow for a better comprehension of the disease's ecology. In close cooperation with partner institutes, insights from other workpackages and expert opinions will be used to parameterize epidemiological models, suited to enhance the predictability of the occurrence of defined sub- and patho-types on the basis of the continuously updated datasets. Examples for candidates are results from experimental infection studies, performed to elucidate the role of selected waterbird species as long-distance carriers, as well as observational and ecological information with reference to the potential ability of different bird species to spread avian influenza viruses, gathered by ornithological and ecological partners.

To prepare the exchange of data and information with related initiatives, as for example the wild-bird monitoring in the European Union or the Global Avian Influenza Network for Surveillance (GAINS) of the World Conservation Society (WCS), data structures and coding systems were implemented and designed to preserve compatibility. All results and insights derived from epidemiological analysis will be communicated with stakeholders, using the framework of the envisaged "early warning and assessment system" coordinated by the project management.

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Co-Coordinator: Hagemeijer W, Wetlands International, Ede, The Netherlands
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# ORAL: THE 2006 OUTBREAK OF EQUINE INFECTIOUS ANAEMIA IN IRELAND – NEW INSIGHTS INTO AN OLD DISEASE

CULLINANE, ANN[1]; QUINLIVAN, MICHELLE[1]; NELLY, MAURA[1]

IRISH EQUINE CENTRE[1]

Key words: EIA, diagnosis, control, incubation period,

Equine Infectious Anaemia (EIA) is caused by a lentivirus of the Retroviridae family. The high incidence of outbreaks of EIA in Romania indicates that within the EU there is a risk of virus spreading from Eastern Europe. In 2006 an outbreak occurred in Ireland, the source of which appears to have been contaminated plasma that was imported from Italy. EIA is a Notifiable Disease and more than 1,500 horses were subject to movement restrictions by the Department of Agriculture. Thirty three cases were confirmed by virological testing. Two clusters were identified linked to a veterinary practice and a referral hospital. During the 12 months after the disease was identified our laboratory tested over 57,000 samples for EIA. The comparative sensitivity of the agar gel immunodiffusion (AGID), four different ELISAs and the immunoblot was evaluated with diagnostic samples from both clinical and subclinical cases. The nucleotide sequence of the gag gene of this virulent strain of EIA virus (EIAV) was determined and two specific and sensitive real time PCR and RT-PCR assays were developed. Viral RNA and DNA were detected by RT-PCR and PCR respectively in all post mortem tissues examined from infected animals. Viral RNA and DNA were also detected in nasal secretions, genital swabs and saliva (RNA only) for the first time. Horses remain EIAV carriers for life thus the detection of seropositive horses and their removal from the population is the basis of effective disease control and eradication. Control measures within the EU are based on an incubation period of up to 90 days and in the United States on a period of 60 days. The serological data from the Irish outbreak suggests that the usual incubation period for this strain of EIAV is approximately 37 days but may be longer than 60 days in a minority of cases. One horse had a possible incubation period of between 120 and 157 days. The data accumulated during this outbreak has important implications for the prevention, control and eradication of a Notifiable Disease.

# ORAL: NOVEL EXPOSURE PATHWAYS FOR POULTRY TO H5N1 HPAI VIRUS FROM WILD BIRDS AND POULTRY PRODUCTS

GALE, PAUL[1]; MUNYINYI, DAVID[1]; KOSMIDER, ROWENA[1]; COOK, ALEX[1]; IRVINE, RICHARD[1]; BREED, ANDREW[1]

VETERINARY LABORATORIES AGENCY (VLA)[1]

Key words: Risk assessment, exposure, pathways, avian influenza virus, H5N1 HPAI

1.Introduction and Objectives

The exposure pathways presented here form part of a qualitative risk assessment to rank the risks of exposure of poultry to H5N1 highly pathogenic avian influenza (HPAI) virus in different sections of the poultry industry in Great Britain (GB), Italy (IT) and the Netherlands (NL), given its release through wild birds or imported poultry meat and products.

2.Material and Methods

The release, exposure and consequence framework according to OIE (2004) was followed. The routes of release of H5N1 HPAI into GB, IT and the NL that were considered included wild birds and illegally and legally imported meat/products. Exposure pathways were constructed based on epidemiological evidence and consideration of the possible fates of wild birds, and routes of dissemination of meat and products. Quantitative data were gathered to populate the pathways, including viral loads in poultry tissues, virus shedding data and decay rates of the virus in water, fomite surfaces and poultry manures.

3.Results

Exposure pathways will be presented for the transmission of H5N1 HPAI from wild birds and other wild animals, which may gain access to backyard and commercial flocks. Environmental pathways, through persistence or dissemination of virus in faeces or pharyngeal secretions are considered as well as routes involving companion animals (cats), domestic livestock (pigs) and insects. Results have been analysed to rank the risks to different poultry sectors through each route, exploring differences, if any, between the three countries.

4.Discussion and Conclusions

The exposure pathways we have investigated represent those already known through epidemiological investigation of H5N1 HPAI outbreaks in poultry, together with those identified through consideration of the possible fates of infected material including wild bird carcasses, infected viscera/tissues, respiratory secretions, faeces and poultry products. The role of rodents, for example, is potentially important since they have been shown to move the carcases of dead birds. This approach may identify new routes, such that the risks can be assessed and managed.

5.Acknowledgements

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6. References

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EPIZONE

# Poster presentations

# POSTER: DEVELOPMENT AND EVALUATION OF VP3-ELISA FOR DETECTION OF GOOSE AND MUSCOVY DUCK PARVOVIRUS ANTIBODIES

ZHANG, YUN [1]

HARBIN VETERINARY RESEARCH INSTITUTE (HVRI)[1]

Key words: Goose parvovirus; Muscovy duck parvovirus; VP3 protein; ELISA;

The VP3-encoding gene of goose parvovirus (GPV) Ep22 strain was cloned and expressed in Escherichia coli. The VP3-encoding gene of GPV comprised 1605 bp, and encoded 534 amino acids with a predicted molecular mass of 59.9 kDa. The expressed VP3 fusion protein in E. coli could be detected by goose and Muscovy duck anti-parvovirus polyclonal serum. In addition, an ELISA (VP3-ELISA) using the expressed VP3 protein as coating antigen for detection of antibodies to GPV in geese and antibodies to Muscovy duck parvovirus (MDPV) in Muscovy ducks was developed. In comparison with the virus neutralization test, the specificity and sensitivity of VP3-ELISA showed 92.0% and 94.4% for goose sera and 93.6% and 96.9% for Muscovy duck sera, respectively. The VP3-ELISA did not react with the antisera to other goose or duck pathogens, implying that this protein was specific in recognition of goose or duck anti-parvovirus antibodies. The cross-reactivity between immunoglobulin G antibodies of goose and Muscovy duck were also tested, reflecting the phylogenetic distance between these birds, and also suggesting it would be more sensitive using the same bird IgG conjugate when employing the ELISA. Taken together, the results demonstrated that VP3-ELISA was a sensitive, specific, and rapid method for detecting antibodies to GPV and MDPV.

# POSTER: CLIMATIC AND LAND USE VARIABLES ASSOCIATED WITH THE BLUETONGUE VECTORS CULICOIDES OBSOLETUS COMPLEX AND CULICOIDES DEWULFI DISTRIBUTION IN PENINSULAR SPAIN

CALVETE, C[1]; ESTRADA, R[2]; MIRANDA, M A[3]; CALAVIA, R[1]; DEL RÍO, R[3]; BORRÁS, D[4]; CALVO, J H [1]; LUCIENTES, J[2]

CENTRO DE INVESTIGACIÓN Y TECNOLOGÍA AGROALIMENTARIA DE ARAGÓN[1]; UNIVERSIDAD DE ZARAGOZA[2]; UNIVERSIDAD ISLAS BALEARES[3]; INSTITUTO DE BIOLOGIA ANIMAL, ISLAS BALEARES[4]

Key words: Bluetongue; Climate; Land-use; Spain; Vector distribution

Bluetongue is a viral disease that is mainly transmitted among their vertebrate hosts (mainly ruminants) by several species of biting midges of the genus Culicoides (Diptera: Ceratopogonidae). Currently, bluetongue is still spreading across peninsular Spain where species of the Culicoides obsoletus complex, probably in association with Culicoides dewulfi, are the main vector species incriminated in viral transmission in the north of the country. Morphological differentiation of these species is difficult since females (the most frequently specimens trapped) of species of the C. obsoletus complex, which are C. obsoletus s.s. and C. scoticus, are indistinguishable and hardly separable from females of C. dewulfi. In consequence, usually both C. dewulfi and species of the C. obsoletus complex are not differentiated and all are considered a single entity from an epidemiological point of view
Given the apparent high affinity of these species regarding climatic conditions, modeling of spatial distribution of all these species combined has been already performed for Spain, however, no attempt has been performed at single species level. The goal of the present work was, therefore, to examine habitat correlations for individual species in this area.
Culicoides spp. catch data for 2008 were provided by the Spanish Bluetongue national surveillance programme. All captured males were identified at species level by morphological criteria and presence/absence of C. dewulfi and species included in the C. obsoletus complex in each sampled locality was recorded. In a first step, generalized linear models with multinomial error distribution and logit link function were fitted and selected by Akaike's criterion to estimate associations between species presence and seven climatic and 47 land-use variables extracted from Corine land cover database. In a second step, spatial autocorrelation was filtered by introducing second order polynomial expansions of geographical co-ordinates of each locality as predictor variables in the final models.
In all, male data were available from 110 localities. Only two species, C. obsoletus s.s. and C. scoticus were found in 102 and 49 localities respectively, whereas the species C. dewulfi, was found in 22. Spatial distribution of C. dewulfi and C. scoticus highly overlapped distribution of C. obsoletus s.s.. Final models showed that, in comparison with C. obsoletus s.s., occurrence of C. dewulfi and C. scoticus was directly associated with land surface occupied by broad-leaved forests and pastures and inversely with precipitation seasonality. In addition, C. dewulfi occurrence was higher when surface occupied by vineyards increased. When comparisons between C. dewulfi and C. scoticus were performed, the occurrence of C. dewulfi was also higher when surface occupied by vineyards increased. When spatial autocorrelation was filtered, only the surface occupied by vineyards kept the statistical significance and the same association with species occurrences.
The statistical associations found suggested that despite the apparently climatic conditions affinity of these Culicoides species and their overlapped distribution, their spatial occurrence might be conditioned by habitat factors, others than climatic conditions, acting at meso-or microhabitat scale. This way, the statistical relationship found between species occurrence and vineyards might be related to differences in soil composition or, more probably, the shortage of moist decaying vegetative material adequate to C. obsoletus s.s. and C. scoticus breeding.

# POSTER: INTRAMUSCULAR IMMUNIZATION WITH ACTINOBACILLUS PLEUROPNEUMONIAE GHOSTS PROVIDES BETTER PROTECTION THAN INTRANASAL IMMUNIZATION TO PIGS AGAINST HOMOLOGOUS CHALLENGE

W, CHUNLAI[1]; L, SIGUO[1]; C, YUEHONG[1]; W, SI[1]; L, HUIFANG[1]; F, CHAOYANG[1]; K, XIANGANG[1]

HARBIN VETERINARY RESEARCH INSTITUTE (HVRI)[1]

Key words: Actinobacillus pleuropneumoniae; ghosts; vaccination; intramuscular; intranasal

Bacterial ghosts are empty cell envelopes achieved by the expression of cloned lysis gene E of bacteriophage PhiX174 and, since there are no denaturing steps during their production, ghosts share functional and antigenic determinants of the envelope with their replicating counterparts, these properties may lead to a superior presentation of surface antigens to the immune system. In the present study, the immunogenic and protective potential of intramuscular or intranasal immunization with ghosts made from A. pleuropneumoniae serotype 1 reference strain Shope 4074 were evaluated. Pigs were vaccinated twice intramuscularly(IMPs) or intranasal(INPs) with a dose of 5×109 CFU ghosts. After 2 weeks vaccinated pigs and non-vaccinated placebo controls (PCs) were challenged with a dose of 109 CFU of A. pleuropneumoniae serotype 1 reference strain Shope 4074 by drops into nasal cavities. The protective efficacy of immunization was evaluated by clinical, bacteriological, serological and post-mortem examinations. The antibody response in serum was determined by ELISAs based on whole-cell antigen. After challenge PCs, IMPs and INPs all developed fever and pleuropneumonia, all PCs and one of INPs died 3 days after challenge. The best protection was observed in IM vaccination group which showed no death after challenge, the lung lesions in IMPs were slighter than those in PCs and INPs, the challenge strain was less re-isolated from lung and tonsils in IMPs compared to PCs and INPs and, the serum antibody titers of IMPs were significantly higher than those of INPs and PCs ($p < 0.01$) two weeks after the second immunization. The results showed that intramuscular immunization with Actinobacillus pleuropneumoniae ghosts can induce better immune protection than that induced by intranasal immunization.

# POSTER: EFFICACY OF INACTIVATED VACCINES AGAINST BLUETONGUE VIRUS SEROTYPE 8 IN SHEEP

ESCHBAUMER, MICHAEL[1]; HOFFMANN, BERND[1]; KÖNIG, PATRICIA[1]; TEIFKE, JENS P.[1]; GETHMANN, JÖRN M.[1]; CONRATHS, FRANZ J.[1]; METTENLEITER , THOMAS C.[1]; BEER, MARTIN[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: bluetongue 8 sheep inactivated vaccine

Bluetongue virus (BTV), an arthropod-borne orbivirus in the family Reoviridae, is a major pathogen of ruminants and camelids that can cause severe disease particularly in sheep. In recent years, bluetongue disease (BT) has become a major animal health problem in the European Union, prompting the member states to establish a vaccination strategy. Monovalent inactivated vaccines against bluetongue virus serotype 8 (BTV-8) by three manufacturers were selected for the compulsory vaccination program carried out in Germany in 2008. The safety of these vaccines was evaluated in a pilot study in the German federal state of Mecklenburg-Western Pomerania. Using animals from that study that had been vaccinated under field conditions, vaccine efficacy was evaluated by serological, virological and clinical examination before and after experimental challenge infection with a German BTV-8 field isolate. Before challenge, antibody levels differed between the vaccinated groups, but all seroconverted animals were fully protected against challenge infection, with no clinical symptoms or detectable virus replication. However, one seronegative sheep in one of the vaccine groups was not protected. One sheep with detectable pre-challenge antibodies was weakly positive in the real-time RT-PCR in a sample taken at day 10 after challenge infection.

# POSTER: PPR VIRUS INFECTION ON SHEEP IN BLACKSEA REGION OF TURKEY:EPIDEMIOLOGY AND DIAGNOSIS BY RT-PCR AND VIRUS ISOLATION

ALBAYRAK, HARUN[1]; ALKAN, FERAY[2]

SAMSUN VETERINARY CONTROL AND RESEARCH INSTITUTE[1]; ANKARA UNIVERSITY VETERINARY FACULTY[2]

Key words: C-ELISA - Sheep - PPRV - RT-PCR - Virus isolation

In this study, a totally 164 materials (lung, spleen, lymph node, nasal and ocular swap, blood and samples from oral lesions) from sheep and lambs (n = 57) in the 34 flocks suspected the PPRV infection as clinically and macroscopic pathologic remarks, housed in the 4 different in the Middle and Eastern Blacksea Region were used for RT-PCR and virus isolation. Additionally, serum samples randomly collected from 892 sheep were tested for the detection of PPRV seroprevalance in the same regions. The seroprevalance were estimated as 14,9% and 3,5–38,2% in the sampled animals and sampled province, respectively. While no virus isolated in Vero cell cultures, PPRV nucleic acid was detected in 26 of 164 materials by RT-PCR. According to the result of RTPCR, the PPRV infection were diagnosed in 44,1% (15/34) and 31,5% (18/57) of the flocks and sampled animals, respectively. Diagnostic value of necropsy materials such as lymph node, spleen, lung and of clinical samples such as nasal swap and conjunctival swap were determined more valuable diagnostic materials in the diagnosis of PPRV infection by RT-PCR. Data showed that PPRV infection was widespread in the Middle and East Blacksea Region and that the prevalence of the infection in the region varies in accordance with the factors such as geographical conditions (climate, etc.) and the method of breeding. Additionally, it is determined that RT-PCR is sensitive and reliable method in the diagnosis of PPRV infection.

# POSTER: FIRST STUDY OF VIRAL HAEMORRHAGIC SEPTISEMIA (VHS) VIRUS IN FRESH WATER FARMED RAINBOW TROUT (ONCORHYNCHUS MYKISS) IN TURKEY

ALBAYRAK, HARUN[1]

SAMSUN VETERINARY CONTROL AND RESEARCH INSTITUTE[1]

Key words: Rainbow trout. Viral haemorrhagic septicemia. RT-PCR. Ag-ELISA. CPE

In order to analyse the presence of viral haemorrhagic septicaemia virus (VHSV) in the fresh water in Blacksea Region in Turkey, fish tissue samples were collected in 2006 and 2007. The sampling comprised 229 samples totalling 473 rainbow trout. All tissue extracts were passaged in RTG-2 (Rainbow trout gonad) cell cultures for two times and cell culture supernatants from all passages and tissue extracts were examined for viral nucleic acid and antigen of VHSV using ELISA (enzyme-linked immunosorbent assay) and RT-PCR (reverse-transcription polymerase chain reaction). No virus was isolated in the isolation studies. The supernatants from passage and tissue extracts were checked nucleic acid and viral antigen of VHSV by RT-PCR and Ag-ELISA. Neither nucleic acid nor viral antigen was detected from cell culture supernatants and tissue extracts.

# POSTER: DIVA (DIFFERENTIATING INFECTED AND VACCINATED ANIMALS) STRATEGIES AND EFFICIENT VACCINES: ADJUVANT INPUT

DUPUIS, LAURENT[1]; DEVILLE, SÉBASTIEN[1]; BERTRAND, FRANÇOIS[1]; GAUCHERON, JÉROME[1]; BENSABER, AMINA[1]; AUCOUTURIER, JEROME[1]

SEPPIC[1]

Key words: Montanide, Adjuvant, DIVA

International trade of live animals is regulated by national and international rules. Presence of several pathogens can be a reason to forbid the trade and importation of animals or even animal product. Recent examples of diseases having induced trade restriction are avian influenza, foot and mouth diseases (FMD) and more recently bluetongue disease in Europe. These diseases can be efficiently prevented by vaccination, but the presence of antibodies in animal blood is also often used as a marker for diagnosis. Therefore a need to differentiate the animal infected from vaccinated is urgent. Selection of potent adjuvant can be a key decision triggering an immune response that will be used to differentiate the vaccinated animals.

Several strategies have been used to allow differentiating infected versus vaccinated animals. In avian influenza, hemagglutinin (H) and neuraminidase (N) antigen are strains specific. Heterologous N strain was used as vaccine antigen to face a local problem and allow differentiating the animal vaccinated versus infected using the immune response directed to the N antigen. In the bluetongue infection that nowadays spread in Europe, some strategies of virus like particles were tested. Those synthetic viral capsides can be associated with antigens which are not present in the wild virus form. Animals vaccinated will therefore develop an immune response to those antigens that infected animal will not present. The FMD case is advanced in the animal identification: a recent Brazilian regulation reduced the authorized level of non structural protein (NSP) that is present in the antigens used for vaccine formulation. Purification process needed to reach this level of NSP clearance induced a drastic loss of antigen properties to induce an antibody response. According to the production batch a reduction 15 to 20% of antibody production was observed with those highly purified antigens.
Therefore specific adjuvants are needed to improve the immune response directed against those antigen allowing DIVA strategies. In avian species, Montanide™ ISA 70 VG showed in avian H5N1 influenza experiment in ducks and poultry the ability to induce much higher antibody titres than the protective 4 log 2 thresholds. In bluetongue disease vaccines, trials using VLP were performed in small ruminants using water in oil adjuvant (Montanide™ ISA 50V2) as adjuvant to promote an efficient and long lasting antibody production. At last, most of vaccines used in field in South America are based on water in mineral oil formulation. The intense immune response induced by such adjuvant formulation is the only one allowing to be complying with the new regulation in NSP purity.
Added to all those strategies, new adjuvant formulations allowing identifying the animals vaccinated without specifically developed antigenic media are underdevelopment. Those formulations are based on an immune stimulation induced by the adjuvant formulation even in absence of antigen.

New vaccines will allow safe animal trade without sanitary restriction, even from endemic area. Those new tools will help animal breeders to develop their market share worldwide.

# POSTER: RELEVANCE OF DUVAXYN® WNV IN THE PREVENTION AND CONTROL OF WEST NILE VIRUS IN THE EUROPEAN UNION

KUMAR, MAHESH[1]; BARNES, HELEN[1]; CHIANG, YU-WEI[1]

FORT DODGE ANIMAL HEALTH[1]

Key words: West Nile Virus, Duvaxyn, Vaccine

West Nile virus is a flavivirus transmitted by the mosquito vector (Culex pipiens and Culex modestus). Hybrid forms of these vectors are known to readily bite both birds and animals. While, birds, horses and humans are commonly infected, the emotional and economical consequences can be very high for horses and humans. Infections can be debilitating and may result in death.

While sporadic incidents of West Nile virus have been reported over the years, in the EU, it remains a disease of major public health concern. The rapid spread in the USA after the initial outbreak and its current epidemic state is significant. The wide use of vaccines has been largely credited with the control of the disease in horses in the USA.

Changing weather patterns and the increase in mosquito vectors have created an opportune time for a similar outbreak in the EU. The recent outbreak in Italy has resulted in the declaration of an endemic state in the region. The recent availability of a vaccine against West Nile virus in the EU, one that contains an antigen from the same lineage of viruses present in the EU, means there is now an effective means for the control and prevention of this important emerging disease.

# POSTER: CHARACTERIZATION OF H10N4 INFLUENZA A VIRUS ISOLATED FROM AVIAN SPECIES AS WELL AS FROM A MAMMALIAN SPECIES, THE MINK

METREVELI, GIORGI[1]; ZOHARI, SIAMAK[1]; STAHL, KARL[1]; BELAK, SANDOR[1]; BERG, MIKAEL[2]

NATIONAL VETERINARY INSTITUTE (SVA)[1]; SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES[2]

Key words: Avian Influenza, wiled birds, mink

Influenza A viruses infect and cause disease in many species. The largest reservoir of these viruses exists in migratory waterfowl, particularly ducks and shorebirds, and include all known antigenic subtypes of influenza A. These usually do not cause obvious disease but are excreted in high titers in feces, thereby facilitating transmission in the natural setting. In 1984, a H10N4 avian influenza virus caused an epidemic outbreak at 33 mink farms. In this outbreak approximately 100 000 minks were affected on the south-east coast of Sweden. This was the first example of a disease in mammals caused by the H10 subtype and the virus was shown to have been of avian origin. We characterized the full genome sequence of the A/mink/Sweden/3900/84 strain together with four other H10N4 viruses isolated in the same period of time in Great Britain, as well as the prototype H10 virus A/chicken/Germany/N/49 (H10N7).Phylogenetic analyses of the sequences of all eight viral RNA segments of A/mink/Sweden/1984 isolate demonstrated that these are wholly avian influenza viruses of the Eurasian lineage. The haemagglutinin gene of the mink isolate fall into same clade as viruses circulated over the same time period in Europe. The identity of the mink nucleotide sequences to those of A/chicken/Germany/N/49 ranged between 89-95% (PB1, PB2, PA, HA, NA, NP, M) and 65% (NS).

# POSTER: EVOLUTION OF LOW PATHOGENIC AVIAN INFLUENZA H7N7 IN MALLARDS IN NORTHERN EUROPE

ZOHARI, SIAMAK[1]; METREVELI, GIORGI[1]; BERG, MIKAEL[2]

NATIONAL VETERINARY INSTITUTE (SVA)[1]; SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES[2]

Key words: Avian influenza, evolution, mallard

Avian influenza viruses of subtype H7 have been responsible for major outbreaks in poultry in the past ten years. Since the first confirmed outbreak of the highly pathogenic avian influenza (HPAI) caused by H7 viruses in England in 1963, this subtype shown to be responsible for more than 15 HPAI outbreaks worldwide, of which outbreaks of 1999 in Italy (H7N1), 2002 in Chile (H7N3), 2003 in The Netherlands (H7N7) and 2004 in Canada (H7N3) clearly demonstrated the potential risk of these viruses to pose a major threat to animal and public health. In this study, we have characterized the whole genome of three avian H7N7 influenza A viruses, isolated from healthy migratory mallards in Northern Europe in three different years, to study the evolution of these viruses in the natural reservoir. Phylogenetic analysis demonstrated that the H7 haemagglutinin genes of studied viruses were all closely related to recent H7 isolates responsible for outbreaks in domestic poultry in Europe. The A/mallard/Sweden/S90735/2003 isolate fell in to separate clade as the HA gene of (H7N3, CY014718.1) virus, isolated from mallard in 2000 in The Netherlands and formed a sister clade with the Dutch H7N7 responsible for HPAI outbreaks in The Netherlands and Germany in 2003. Whereas the HA gene of the two mallards strain A/mallard/Sweden/S90597/2005 and A/mallard/Sweden/100993/2008 were more related to the strains isolated in domestic poultry in England in 2006 and 2008, respectively (LPAI-H7N3 , EF467826.1 and HPAI-H7N7, FJ476173). Nucleotide sequence identities of HA gene within the studied viruses were 96 to 98 percent, with the largest divergence (2%) found between A/mallard/Sweden/S90735/2003 and A/mallard/Sweden/100993/2008. Analysis of the deduced HA amino acid sequence shows two different HA cleavage sites of these isolates. Although these HA cleavage sites are consistent with a low pathogenic AI, the cleavage sites appear to evolve into a more HP type during time (PEIPKGRGLF in 2003 and 2005 and PEIPKKRGLF in 2008). The other genes were very similar and all belong to the Eurasian avian lineage. The conclusion from this study is that H7 subtypes isolated from healthy mallards are closely related to H7 subtypes causing outbreaks in poultry in Europe and these viruses continue to pose great threat to animal health.

Acknowledgements
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# POSTER: DEVELOPMENT OF A PRIMER-PROBE ENERGY TRANSFER REAL-TIME PCR ASSAY FOR THE IMPROVED DETECTION OF CLASSICAL SWINE FEVER VIRUS

LIU, LIHONG[1]; XIA, HONGYAN[2]; BELAK, SANDOR[1]; WIDEN, FREDERIK [1]

NATIONAL VETERINARY INSTITUTE (SVA)[1]; SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES[2]

Key words: CSFV, C-strain vaccine, real-time PCR assay

Classical swine fever (CSF) is a highly contagious and devastating disease, causing serious losses in the pig industry worldwide. Vaccination of pigs with the conventional C-strain vaccine has been practiced in different regions of the world in order to prevent the disease. In the control programmes of CSF, rapid detection and identification of the causing agent Classical swine fever virus (CSFV) is a crucial step. Here, we report a novel real-time PCR assay based on primer-probe energy transfer technology for detection of CSFV. The assay is able to detect 20 copies of viral cDNA per reaction, showing a high sensitivity. The specificity has been evaluated by testing 58 pestiviruses, representing all species and unclassified pestiviruses. The assay has been found highly reproducible. Following PCR amplificaiton, the melting curve analysis allows confirmation of specific amplicons, and differentiation between wild type CSFV and certain C-strain vaccines. It could be used for the improved routine laboratory diagnosis of CSF.

# POSTER: MOLECULAR DETECTION OF PATHOGENS RESPONSIBLE FOR PROGRESSIVE ATROPHIC RHINITIS

MARKOWSKA-DANIEL, IWONA[1]; STEPNIEWSKA, KATARZYNA[1]; URBANIAK, KINGA[1]; PEJSAK, ZYGMUNT[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: nasal swabs, Pm, Bbr, PCR

Introduction

Atrophic rhinitis caused by toxigenic strains of Pasteurella multocida (Pm) and Bordetella bronchiseptica (Bbr), is highly prevalent, economically significant disease. Pm and Bbr can be detected by the microbiological methods. To evidence the presence of dermonecrotoxin (DNT) more accurate method is PCR. In this study the results of PCRs, evaluated for detection of Pm' and Bbr' DNT, directly in clinical material, are presented.

Material and methods

A total of 289 pigs' nasal swabs from 22 farms located in Poland, were tested. Samples were taken from pigs which do not demonstrated typical clinical signs of the disease. Swabs were analyzed by direct PCRs and standard microbiological methods. For detection of DNT Pm the pair of primers which amplified fragment of 501bp, were used. For detection of DNT Bbr the set of primers designed by ourselves, which amplified fragment of 224bp were used. Cultures were performed using agar supplemented with 5% of horse blood (for Pm) and G20G medium (for multiplication of Bbr). Plates were incubated at 370C for 24h (for Pm) or 48-72h (for Bbr).

Results

In bacteriological examination Pm was detected in 70 swabs (24.2% of tested samples), taken from pigs raised in 4 out of 22 suspected farms (18.2%). Bbr was evidenced in 114 samples (39.4% of examined swabs), obtained from 15 farms (68.2%). In molecular testing the presence of DNT Pm was detected in 60 swabs (20.7% of samples). On the farm level DNT Pm was evidenced in 10 out of 22 farms (45%). The percent of samples positive for the presence of DNT Bbr was equal to 47.4 (137 swabs). DNT Bbr was detected in 18 farms (81.8%).

Discussion

Presented results show that both PCR tests were useful for detection of DNT Pm and Bbr directly in nasal swabs. The detection of DNT Pm based on the paper published by Register et al (2). Mentioned authors use the technique for detection of DNT in pure culture only, not in clinical material, as we did after modification of the original protocol. This modification makes the diagnosis of PAR cheaper and at least 1 day quicker which is important factor from the diagnostic and practical point of view. It should be underlined that molecular methods used in the experiment were much more precise than classical ones. For example Pm was isolated in 24.2% of tested swabs but most of the isolates (50, which is equal to 71.4%) do not possessed the gene encoding DNT. Additionally Pm was isolated only in 4 farms while DNT Pm was evidenced in 10 farms. Bbr was detected in 4 farms only by PCR. Comparing the results of culture and PCR it was evidenced that using PCR in 5 farms we detected 20% more samples, in 3 farms - 10%, in 2 farms - 30%, in 1 farm - 50% and in 1 farm 70% in comparison to the bacteriological technique. It might result from the fact that culture methods depend strictly on time between sampling/examination and transport conditions as well as concentration of the bacteria and incubation period for their grow, as it was evidenced by Lariveire S. et al (1) as well as by us in the previous study. Summarizing, diagnosis of PAR based on testing of nasal swabs by PCR is faster, more sensitive, specific and accurate that long-term cultivation used in classical bacteriology.

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* Paper prepared within Project No N308 3228 33

# POSTER: HOW DO WE DETECT NOVEL VIRUSES USING MICROARRAYS?

WATSON, MICHAEL[1]

INSTITUTE FOR ANIMAL HEALTH (IAH)[1]

Key words: Novel virus detect microarray detectiv diagnostic

Microarrays have provided a new method of performing thousands of diagnostic assays in a single experiment, and have the potential to revolutionise this field. However, the data is not without problems, including non-specific hybridisation, specific cross-hybridisation and "rogue probes".

So what is the place of these arrays in the diagnostic laboratory?

Many labs throughout the World have reported the discovery of novel viruses using microarrays. Where that data is publicly available, I will present to you the evidence using DetectiV [1], and thus I will show what a novel virus looks like on an array that was designed to detect known viruses. I will show that, far from being clear, the evidence often points to an existing virus, or is statistically no different to background data. So how is that scientists concluded the presence of a novel virus?

I will discuss the role of microarrays alongside other technologies, and finally present future ideas for designing array based diagnostic tests using next-generation arrays.

[1] Watson M, Dukes J, Abu-Median AB, King DP, Britton P.(2007) DetectiV: visualization, normalization and significance testing for pathogen-detection microarray data. Genome Biol. 8(9):R190.

# POSTER: DIFFERENTIATION BETWEEN CYPRINID HERPESVIRUS TYPE-3 LINEAGES USING DUPLEX PCR

BIGARRE, LAURENT[1]; BAUD, MARINE[1]; CABON, JOELLE[1]; ANTYCHOWICZ, JERZY[2]; BERGMANN, S. M. [3]; ENGELSMA, MARC[4]; POZET, FRANÇOISE[5]; REICHART, MICHAL[2]; CASTRIC, JEANNETTE[6]

AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[1]; NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[2]; FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[3]; CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[4]; LABORATOIRE DÉPARTEMENTAL D'ANALYSES DU JURA[5]; AGENCE FRANÇAISE DE SECURITÉ SANITAIRE DES ALIMENTS PVP (AFSSA)[6]

Key words: Alloherpesviridae, KHV, CyHV3, carp, duplex PCR

To date, all the isolates of Cyprinid herpesvirus type-3 (CyHV3) responsible for serious outbreaks in carps Cyprinus carpio have been found to be very similar or identical on the basis of DNA sequences of a few reference genes. However, two genetic lineages (U/I and J) are distinguished by full-length genome sequencing. Two molecular markers presenting genetic variations were targeted for developing a duplex PCR assay able to distinguish CyHV3-U/I from CyHV3-J while avoiding DNA sequencing. The method was validated on a series of 42 samples of infected carps from France, The Netherlands and Poland collected from 2001 to 2008. Among these samples, both the U/I and J genotypes were identified, but also a third genotype representing a genetic intermediate between U/I and J for one of the two molecular markers. A classification of CyHV3 genotypes, based on the alleles of the two molecular markers, is proposed. The assay is easy to perform and provides a genotype information with samples moderately or highly concentrated. This tool should improve our knowledge regarding the present distribution and future diversification of this emerging virus.

# POSTER: AN ORAL, LOW-DOSE INTERFERON-ALPHA TREATMENT CAN MODULATE THE STRESS OF EARLY WEANING IN PIGS

AMADORI, MASSIMO[1]; FARINACCI, MAURA[2]; COLITTI, MONICA[2]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL' EMILIA ROMAGNA (IZSLER)[1]; FACULTY OF VETERINARY MEDICINE[2]

Key words: pig; stress; weaning ; interferon-alpha; inflammatory response.

Interferon-alpha (IFN-alpha) at low concentrations had been previously shown to control in vitro the expression of inflammatory cytokine genes in swine pulmonary alveolar macrophages. Owing to the above, we wondered if a similar control action could be exerted by IFN-alpha in piglets during a natural stressing event like early weaning, characterized by a high proflogistic potential. To this purpose, four Specific Pathogen Free (SPF) littermate piglets were given human lymphoblastoid interferon-alpha for 10 days in a row in form of a freeze-dried preparation, mixed in the diet, starting on the day of early weaning at 21 days of age. The other four littermate piglets served as untreated controls; they received the same daily mass of placebo in a separate weaning cage. The expression of IFN-alpha, IFN-gamma, IL-1 beta, TNF-alpha and IL-6 genes was determined in uncultured PBMC by real time PCR. Orally administered IFN-alpha was shown to reduce after weaning the expression of the IFN-gamma gene ($p<0.08$) and to increase that of the IL-1 beta gene ($p<0.05$). There was also a trend to a reduced expression of both IL-6 and TNF-alpha genes. Relatively to the day of weaning, endogenous IFN-alpha was demonstrated in sera and also PBMC of untreated, control piglets at day +3, as opposed to day –1. The above modulation of cytokine gene expression and the greater daily mean weight gain of IFN-treated piglets highlight important regulatory properties of oral IFN-alpha treatment at weaning, which probably resemble those of the endogenous cytokine. A greater daily mean weight gain and significant changes in the time-course of the main inflammatory cytokines in sera were also shown in a field trial of oral, low-dose IFN-alpha treatment at weaning.

# POSTER: EARLY PRIMING OF T-CELLS WITH THE ATTENUATED VIRAL VACCINE - IMPLICATION TO CELL-MEDIATED IMMUNITY OF PIGS*

POMORSKA-MOL, M[1]; MARKOWSKA-DANIEL, I[1]; KOWALCZYK, A[1]; STEPNIEWSKA, K[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: pigs, vaccination, cellular immunity, maternal antibodies

The level of antibodies is often not correlated with protection against the challenge. Cell mediated immunity is more important for viral clearance, as it was demonstrated for Aujeszky's disease virus (ADV) (1). The presence of maternal immunity at the time of vaccination is disadvantage, as it interferes with the induction of an active immune response after vaccination (2). The efficiency of early vaccination with attenuated ADV in the presence of maternal antibodies were investigated after vaccination of sows and piglets using different schedule.

Six seronegative pregnant sows and their litters were used. For immunization of animals attenuated vaccine against AD was used. Sows were vaccinated twice before partum. Piglets from group 1 were vaccinated once (at 8 weeks of age), from group 2 - twice (at 7 days and 8 weeks of age) and from group 3 - served as unvaccinated control.
Blood samples were taken before and two weeks after each vaccination. To evaluate the cellular response the proliferation assay as well as analysis of co-expression of CD25 with CD2 and CD8 on lymphocytes stimulating in vitro by live ADV were used. Porcine PBMCs were cultured with or without ADV for 72 h before cytometric analyses. For proliferation assay the cultures were pulsed for final 18 h with [3H]-thymidine. The radioactivity incorporated was measured as counts per minute (cpm).

An increase of SI value above 3.0 was observed in 50% of piglets vaccinated at 7 days of age. High values of SI were also noted in group 1 (60% animals) and group 2 (all animals). In unvaccinated groups the mean SI value was 0.9 Following the exposure to the ADV total CD25 expression on PBMCs (including CD2+ and CD2-cells) slightly increased in group 1 and 2, whereas in group 1 the decrease of such expression was evidenced. On PBMCs obtained from animals vaccinated twice, the increase of CD25 expression on CD2+ cells was statistically significant ($p \leq 0.1$) in comparison to non stimulated control. In this group significant increase of percentage of cells with CD2+ and CD8+ phenotype were also observed ($p \leq 0.1$). In group 2 the index of CD4+/CD8+ were lower in cells stimulated with ADV than in non-stimulated control as well as in the groups 1 and 3.

Our results indicate that vaccination of piglets as young as 7 days of age can successfully priming of T lymphocyte. After booster dose all animals were shown antigen-specific response in proliferation test, whereas in pigs vaccinated once, at 7 days or 8 weeks of life, only 50 and 60% of animals, respectively, reacted. The SI value above 3.0 was considered positive for antigen-specific proliferation. Surface expression of IL-2R-alpha (CD25) is widely used to identify activated lymphocyte populations. In our study only in group 2 the significant increase of activated PBMCs, CD2+ and CD8+ phenotype cells were noted in comparison to non-stimulated control. There are no increasing percentage of CD2-CD25+ cells (including B-cells and γδT cells) in all tested groups after ADV stimulation.
Decreasing of CD4+/CD8+ index in group 2 was caused by increasing of the percentage of cytotoxic T-cells in stimulated culture, which can indicate antigen-specific response of these cells. Thus, ADV sensitization of lymphocytes had been evoked by vaccination despite the presence of maternal antibody.

Acknowledgments: The NIA3 ADV strain was kindly provided by Dr A. Lipowski (NVRI Pulawy)

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# POSTER: RESULTS OF THE VACCINATION AGAINST BTV-8 IN GERMANY 2008

GETHMANN, JOERN[1]; PROBST, CAROLINA[1]; BEER, MARTIN[1]; HOFFMANN, BERND[1]; CONRATHS, FRANZ J[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: bluetongue, inactivated vaccines

Introduction

In January 2008, the European Commission decided to implement a mass vaccination program against Bluetongue virus serotype 8 (BTV-8) for cattle, sheep and goats. At that time inactivated vaccines against BTV-8 were not yet available. As the manufacturers could not provide sufficient information regarding the safety of the vaccines, Germany decided to carry out a safety study before starting the compulsory mass vaccination. A precondition for all manufacturers for selling their vaccines in Germany was the participation in this safety study. The study started in March 2008. Three manufacturers provided their monovalent BTV-8 inactivated vaccines: BLUEVAC® 8 (CZ Veterinaria, Spain), BTVPUR® AlSap 8 (Merial, France) and Zulvac® 8 Bovis (Fort Dodge, The Netherlands).
In May 2008 the compulsory vaccination program was launched for cattle, sheep and goats with these three vaccines.

Results

The results of the safety study showed that all three tested vaccines were safe. Only minor reactions like transient swellings or a temporal rise in body temperature were observed. Mass vaccinations were carried out from May to December 2008. Approximately 18 million doses were applied to cattle, about 2.6 million doses used in sheep and about 0.2 million doses in goats (source: HI-Tier, Herkunftssicherungs- und Informationssystem für Tiere, German Identification and Information system for Animals).
The number of BTV-8 outbreaks decreased from more than 20,000 cases in 2007 to about 3,000 cases in 2008. Most cases occurred in a ring-shaped area adjacent to the former epidemic area of 2007.

Discussion and Conclusions

Reporting potential side effects observed after vaccination to the Paul-Ehrlich-Institute was mandatory. An analysis of the pharmacovigilance reports received so far showed that side effects were rare (1-10 cases in10,000 vaccinations) and included mainly abortions or other reproductive disorders. Animal losses were very rare (less than 1 in 10,000 vaccinations) [2]. However, it has to be taken into account that potential causal relationships between the reported events and vaccination were not investigated. These results confirm that the three vaccines are safe. Due to a delay in the availability of the vaccines the immunisations could not start before May 2008. As cattle have to be vaccinated twice in a four weeks interval, a large proportion of the animals was not fully protected until the end of August. For that reason more than 3,000 BT cases occurred despite the compulsory mass vaccination program. However, a high vaccination coverage was achieved until the end of the year in both cattle and sheep.

Outlook

Germany will continue to vaccinate against BTV-8 in 2009. As it is now possible to immunise the animals in winter, they should be protected before the new BT season starts. Therefore a further decrease in the number of new outbreaks is expected.

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# POSTER: OCCURRENCE OF THE HVT AND CVI988 RISPENS VACCINE STRAINS AFTER THE IN OVO AND 1-DAY OLD CHICKENS VACCINATION

WOŹNIAKOWSKI, GRZEGORZ[1]; SAMOREK-SALAMONOWICZ, ELŻBIETA[1]; KOZDRUŃ, WOJCIECH[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: Marek's disease, vaccination, distribution of the vaccine strain virus, FC126 HVT, CVI988 Rispens

Marek's disease is a tumorous disease of chickens, turkeys and Japan quails which causes major losses in mass poultry production. Its etiological agent belongs to Herpesviridae family. The only method of protection against Marek's disease is the vaccination of chickens. The aim of this study was the determination of the localization of HVT FC126 and CVI988 Rispens strains in visceral organs of chickens after vaccination. Two vaccination methods were applied: in ovo vaccination on the 18th day of incubation of chicken embryos and vaccination of 1-day old chickens via the intramuscular route. One hundred SPF chicken embryos were divided into the five groups for 20 embryos. The forty embryos were vaccinated in ovo with a dose of 1400 PFU of vaccine strains. Twenty embryos from the group I were vaccinated with HVT FC126 strain while twenty embryos from group III were vaccinated with CVI 988 (Rispens) strain. The hatched chickens from the non-vaccinated embryos were divided into three groups. On the first day after hatching 20 chickens from group II were vaccinated intramuscularly with HVT FC126 strain with a dose of 1400 PFU and the 20 chickens from group IV were vaccinated intramuscularly with CVI 988 Rispens strain with the same dose. The twenty non-vaccinated chickens from the group V were used as a control. The samples of livers, spleens, bursas of Fabricius, thymuses, lungs and kidneys were taken from embryos belonging to group I and III just before their hatching. The same organs were taken on the 1st, 3rd, 7th, 10th day of life from chickens belonging to all of the groups.

Total DNA was extracted from the samples and used for the detection of the viral DNA with PCR method. For the amplification reaction the primers complementary to SORF1 gene of FC126 MDV-3 serotype and the primers complementary to 132 bp repeated sequence of MDV-1 were used. The signal specific for the HVT FC126 strain was found in DNA samples extracted from bursas of Fabricius, thymuses and spleens of embryos from group I just before their hatching. Among the 1-day old chickens from group I the presence of characteristic PCR product for HVT FC126 strain was detected in bursas of Fabricius, thymuses, livers, spleens, lungs and kidneys. The presence of PCR products characteristic for the CVI988 Rispens strain was found in samples of bursas of Fabricius and lungs taken from the vaccinated embryos from group III just before their hatching. Beginning from the 3rd day of chickens life the presence of PCR product characteristic for FC126 HVT strain was observed in all the samples collected from group I and II while the DNA of CVI988 Rispens strain was found in bursas of Fabricius, thymuses, spleens, lungs and kidneys from chickens belonging to groups III and IV on the 3rd day of chickens life. The results for the 5th group of not-vaccinated chickens were negative.

The obtained results confirmed the previous finding that the in ovo method protects the vaccinated chickens considerably earlier than the vaccination of the 1-day old chickens. The replication of the HVT FC126 vaccine strain of Marek's disease virus originated in the bursa of Fabricius, thymus and spleens then in the rest of the organs. The replication of the CVI988 Rispens strains originate in bursas of Fabricius and lungs and kidneys, then the virus is present in the rest of visceral organs. The results will hopefully help to better understand the efficiency of the in ovo vaccination against Marek's disease and the distribution of the vaccine strains in the visceral organs of chickens after vaccination.

# POSTER: PRELIMINARY STUDY ON OCCURRENCE WEST NILE VIRUS IN POLAND

NICZYPORUK, JOWITA SAMANTA[1]; SAMOREK-SALAMONOWICZ, ELŻBIETA[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: West Nile Virus, Flaviviridae family, arbovirus, ssRNA+, NRT – PCR.

W.P. 6.4 Molecular Epidemiology

West Nile Virus (WNV) is the member of the Flaviviridae family, the Flavivirus spp. It has an icosahedral, spherical capsid, 40-60 nm in diameter. The genetic material of West Nile Virus is a positive-sense, single strand of RNA. WNV genome (10 kb) is coding a single ORF (Open Reading Frame), flanked by short non-coding regions. Virus RNA encodes three structural proteins glycorotein E, membrane protein prM and capsid protein C. WNV also consists of 7 non-structural proteins: NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5.
West Nile Virus can infected humans, birds, mosquitoes, horses, and some other mammals.
The aim of the study was to determine of occurrence West Nile Virus in Poland by method NRT-PCR (Nested Reverse Transcription PCR) and commercial West Nile Virus kit Prodesse.
The 240 wild birds: goshawks (45 birds), buzzards (30 birds), crows (12 birds), ravens (3 birds), wild ducks (122 birds), fulica (17 birds), pheasant (17 birds), and stork (1 bird) were used. Samples of the brain from wild birds were homogenized and whole cell RNA was isolated. Primers for RT-PCR were based on conservative sequence 3'NCR (non-coding region). Primers for Nested-PCR were designed on the basis of RT-PCR amplicon sequence. Thermal conditions for RT PCR were: 50°C for 30 min (initial incubation), 95°C for 15 min, (initial denaturation) 35 cycles; 94°C for 45 s (denaturation), 54°C -45 s (annealing) and 72°C for 1 min; 72°C for 10 min (final elongation). The termal conditions for Nested-PCR were 95°C –for 3 min (initial incubation), 35 cycles 94°C for 45 s (denaturation), 58°C - 45 s (annealing), 72°C - 1 min (elongation) 72°C - 10 min (final elongation). RNA leads to acquiring cDNA from RNA genome and Nested PCR was performd. The results of the reaction of amplification RT PCR and Nested PCR were tested on the 2% agarose gel. Samples were visualized on a UV transilluminator and photographed. DNA product was 150 bp in length.
The West Nile Virus Prodesse ™ Kit first step is reversed transcription and then reaction of amplification. Results are gained by colorimetric assay. Test was performed as commercial protocol indicated. The results with our own method were compared with the results of West Nile Virus Kit Prodesse.
All the samples were examined with NRT PCR and also with commercial West Nile Virus Kit Prodesse™. All positive controls were correct. There was no genetic material of West Nile Virus detected in examinated samples.

# POSTER: MOLECULAR DETECTION OF WEST NILE VIRUS USING REAL-TIME PCR: A COMPARATIVE STUDY

EIDEN, MARTIN[1]; VINETA RODRIGUEZ, ARIEL[1]; ZIEGLER, UTE[1]; GROSCHUP, MARTIN H.[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: WNV, Arbovirus, real-time RT-PCR, diagnosis, surveillance

West Nile virus (WNV) is an arbovirus, and belongs to the genus Flavivirus in the family Flaviviridae, which is transmitted by birds and Culex ssp. WNV was isolated for the first time in Uganda in 1937 and was only found in the Old World until recently. However, in 1999 WNV emerged in the New World, when it was detected during an outbreak of encephalitis in New York City. Since the year 2000 WNV has spread across North America and into Central America. In Europe only spatially (and in most instances also timely) limited WNV outbreaks happened in southern and eastern European regions up to now. The WNV infection in humans is characterised in a mild form by fever, but also in more severe cases by encephalitis accompanied with a meningitis and myelitis. WNV has two genetic lineages: lineage 1 has been isolated in North America, Europe, Africa, Asia and Australia. Lineage 2 comprises WNV strains from sub-Saharan regions of Africa and from Madagascar and more recently in Austria. In this study a new real-time PCR assay for the sensitive detection of WNV strains from lineages 1 and 2 was developed and compared to previously published real-time PCR assays. Primers and probes were designed using multiple alignments of previous and recent WNV sequences and were directed to a highly conserved sequence within the 5'NC region of the WNV genome. Both lineages were detected equally well and with highest efficiency. The new assay will be used for surveillance of WNV in Germany.

# POSTER: SEQUENCING OF THE TWO FIRST IHNV ISOLATES IDENTIFIED IN POLAND

BORZYM, EWA[1]; ANTYCHOWICZ, JERZY [1]; MATRAS, MAREK [1]; REICHERT , MICHAŁ[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: IHNV, sequencing

The first two IHNV isolates were identified in Poland at two different locations. One originated from clinical case of the disease other from the asymptomatic carrier rainbow trouts. They were isolated in EPC and FHM cell lines and identified using commercial Elisa test, RT-PCR test and immunofluorescence method. For these tests RNA extracts of these fish samples were serially diluted in nuclease free water from 10 -1 to 10 -10.
The aim of this study was to conduct a comprehensive phylogenetic analysis of this isolates and investigate their eventual relation to other European IHNV isolates.
For RT-PCR reaction and for sequencing one primer pair amplifying the 697 bp DNA fragment of the G-gene was used. The forward and reverse primer sequences were as follows: 5′-ATGATCACCACTCCGCTGATT-3′ and 5′-GATTGGAGATTTTATCAACA-3′ respectively. The analysis of DNA sequences of Polish IHNV isolates was made, and their similarity to other IHNV variants clustered in European genogroup ware shown.

# POSTER: A SPATIAL DATA INFRASTRUCTURE TO SUPPORT THE SURVEILLANCE PROGRAMMES IN NORTH-EASTERN ITALY

FERRÈ, NICOLA[1]; MAZZUCATO, MATTEO[1]; TROLESE, MATTO[1]; MAZZAGALLO, SILVIA[1]; CIBIN, VERONICA[1]

INSTITUTO ZOOPROFILATTICO SPERIMENTALE VENEZIE[1]

Key words: SDI, surveillance, WebGIS

In 2002 the Veterinary services of the Veneto Region (North-Eastern Italy) developed a WEB-GIS (GeoCREV) that allowed veterinarians from the Local Health Units to directly access geographical data and other relevant information associated to production sites. Throughout the GeoCREV more than 50,000 livestock holdings were indirectly georeferenced and more than 200 contact per year were recorded. The system provided valuable support to the veterinarians and policy makers during the Avian Influenza epidemics (5 different waves from 1999 up to 2005) in the application of territorial measures such as stand-still of animal movements, emergency vaccination, pre-emptive culling policy and identification of areas put under restriction measures.
After 5 years of service the system showed some drawback, mainly linked to the organisational capability. Stakeholders' perception of the system changed from sceptical (or even antagonistic) to highly supportive: their expectations changed in time as they got more familiar with the technology. As a consequence of this new awareness there was an increased request of GIS analyses, applications and layers. The new requirements not always fitted with the system capabilities and, as a consequence, the system was, sometimes unable to answer properly to the requests. Other limitation showed by the system were: (i) differences in formats and quality of data; (ii) redundant maintenance of data sets; (iii) differences in hardware platforms and proprietary software.
In 2008 the Veneto Region developed an action plan for the evolution of the actual CorporateGIS structure of GeoCREV through a Spatial Data Infrastructure (SDI) system. The consensus process started with a survey of the already in place SDIs at national and international level, their structural components and the analysis of the national and international legislation on this matter (Dir.2007/2/EC; INSPIRE). On the basis of the resources available and following a consultancy exercise with the Regional SDI managers, a development programme was initiated. This development programme was splitted into three working groups: (i) information infrastructure; (ii) governance infrastructure; (iii) data management. The development of an information infrastructure involved the development of a WebGIS. The mission of the thematic group for the governance structure development was the establishment of a set of common rules that ensure the interoperability and integration of data with other GIS resources. In particular the thematic group developed some provisions in order to deal with methods, data quality and organisational issues within which providers and users from various organisation and sectors can access the spatial and non-spatial data. The thematic group for data management developed a Data Mart that enables the storage, query and analysis of data managed by the system.
Providing integrated access to multiple distributed heterogeneous spatial data and other information sources is the main goal of the new GeoCREV. This system automatically acquires data through a Virtual Private Network or a set of WEB-Services fed from six different sources: (i) the regional database of poultry holdings; (ii) the Laboratory Information and Management System; (iii) the Animal Disease Notification System and the Outbreak Investigation Database; (iv) the geo-database containing the holding coordinates published through a WEB-GIS system; (v) the base cartography maps form the Veneto regional SDI; (vi) some environmental layers form the environmental department of Veneto Region.

# POSTER: FLOCK-LEVEL SEROPREVALENCE SURVEY FOR SMALL RUMINANTS LENTIVIRUSES IN SEVERAL ITALIAN TERRITORIES

FELIZIANI, FRANCESCO [1]; STANGHELLINI, ELENA [2]; RANALLI, GIOVANNA [2]; GUIDONI, MAIRA[3]; PERUGINI, GIANNI [1]; PONTI, NICOLETTA [4]; PURPARI, GIUSEPPA[5]; RICCI, IDA[3]; GIAMMARIOLI, MONICA[1]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE UMBRIA E MARCHE[1]; UNIVERSITY OF PERUGIA[2]; INSTITUTO ZOOPROFILATTICO SPERIMENTALE LAZIO E TOSCANA[3]; ISTITUTO ZOOPROFILATTICO SPERIMENTALE SARDEGNA[4]; ISTITUTO ZOOPROFILATTICO SPERIMENTALE SICILIA[5]

Key words: prevalence SRLV goats sheeps

Small Ruminant Lentiviruses (SRLV) comprise Maedi Visna Virus of sheep and Caprine Arthritis Encephalitis Virus of goats. These retroviruses cause chronic inflammatory disease in the brain, lungs, joints, and mammary glands. Transmission is mainly via the ingestion of infected colostrum and milk or through respiratory contact. Following infection, animals produce anti-viral antibodies, though seroconversion may occur any time from a few weeks to several months. The animals, after infection, stay infected for their lifetime and serve as a carrier even in the presence of circulating antibodies. Unlike some viral diseases, the presence of antibodies is not indicative of immunity.

Economic losses, due to the occurrence of SRLV infection, can vary considerably between flocks: economic costs may include marketing and export restrictions, premature culling, and losses from poor milk production due to indurative mastitis.

The objectives of this study were to describe the serological prevalence of SRLV in sheep and goats flocks of different Italian territories, and to identify management and demographic variables that would be associated with seroprevalence for this infection.

A sample of 484 sheep and goats flocks was randomly sampled in 7 Italian provinces. The owners of these flocks were surveyed regarding management procedures on their farms, and blood samples were taken from a random and representative sample of animals in each flock. On the basis of tests results, flocks were classified according to seroprevalence: high (>70%), intermediate (30–69%), low (01–29%), and negative (SRLV-free).

At least one subject tested serologically positive, based on the ELISA technique, in 88.9% of the farms. Positive serological reactions occurred in 31.1% of the 21313 animals tested. Flock demographics and farm management variables were considered in a statistical analysis model, and several factors were positively associated with higher SRLV seroprevalence rates. These included the geographical localization of the flock, the bred species and other zoo-technical parameters.

The results of this study shows that SRLV infections is highly widespread in Italian flocks; particularly different seroprevalence level were estimated in the studied territories. According with previous researches factors such as the flock size or flock type can influence the presence of infection: large flocks had higher seroprevalences than smaller ones. This aspect probably agrees with an increase in the horizontal transmission of SRLV by respiratory secretions when there is a high animal density, and it may also be related to traditional management practices.

This study encourage the application of measures to reduce the prevalence of SRLV in Italian flocks: specific control programmes, adapted to rearing system, prevalence and management conditions should be chosen; the determination of disease prevalence is, in fact, the initial action in any eradication scheme. SRLV prevention strategies should be applied in the considered territories, in a continuous manner to progressively eradicate infection. SRLV-free certified flocks should be the final stage and will open interesting economical possibilities for Italian breeds at local and international level.

# POSTER: A DIVA SYSTEM BASED ON THE DETECTION OF ANTIBODIES TO NONSTRUCTURAL PROTEIN 3 (NS3) OF BLUETONGUE VIRUS

BARROS, SILVIA[1]; RAMOS, FERNANDA[1]; CRUZ, BENEDITA[1]; LUIS, TIAGO[1]; FAGULHA, TERESA[1]; DUARTE, MARGARIDA[1]; HENRIQUES, MARGARIDA[1]; FEVEREIRO, MIGUEL[1]

LABORATÓRIO NACIONAL DE INVESTIGAÇÃO VETERINÁRIA[1]

Key words: Bluetongue virus; NS3; ELISA; DIVA

Vaccination is the most effective mean to protect animals against Bluetongue virus (BTV) and to reduce the spread of virus after infection. Inactivated vaccines against serotypes 1, 2, 4, and 8 of BTV were recently developed and have been used in the European Union, replacing the conventional live attenuated vaccines. These vaccines offer significant advantages over attenuated vaccines because of the absence of replicating virus. However, vaccination interferes with the serological detection of infected animals. To overcome this problem we developed a DIVA test (differentiate infected from vaccinated animals) that looks at a differential immune response to the BTV nonstructural protein 3 (NS3). The nonstructural NS3 protein is produced in large quantities in infected cells, but is not packaged in the virion. Since inactivated vaccines for BTV are primarily made with whole virions, a differential antibody response should be observed between naturally infected and vaccinated animals. Therefore, NS3 antibody response is related in vivo to the virus activity.

The NS3 encoding gene of strain BTV4/22045/PT04 was inserted into expression vector pET-28a and expressed in Escherichia coli strain JM109. Recombinant NS3 protein was used as an antigen in an indirect ELISA (NS3-ELISA) to measure the serologic response to NS3 protein in cattle and sheep. Following a vaccination/challenge experiment, with a bivalent inactivated BTV 2-4 vaccine (Merial), seven vaccinated and one unvaccinated control bovine were evaluated for the ability to develop antibodies to NS3. All vaccinated animals were negative by NS3-ELISA and, with exception of one, remained negative even after challenge with BTV4/22045/PT04 while control animal developed NS3 in response to challenge infection. All animals developed VP7 and neutralizing antibodies.

To evaluate the applicability of NS3-ELISA, 562 field serum samples collected from uninfected, vaccinated and from infected ruminants with serotypes 1 or 4 of BTV were screened for NS3 antibodies. Taken together, the results confirm that NS3 antibodies were induced to the greatest levels in ruminants infected with BTV in comparison to the levels induced in vaccinated animals, implying that antibody response to NS3 allows the differentiation between infected and vaccinated animals.

# POSTER: BLUETONGUE PREVENTION IN RUSSIA

ZAKHAROV, VALERY[1]; MISHCHENKO, VLADIMIR[1]

FGI ALL-RUSSIAN RESEARCH INSTITUTE FOR ANIMAL HEALTH[1]

Key words: bluetongue, imported cattle

The bluetongue epidemic situation in Europe in 2008 demonstrated a clear tendency to spread in northern and eastern directions.

A single bluetongue outbreak occurred in Russia in 1993 when one case was registered on a sheep farm in Tapkhar, the Republic of Buryatia. The morbidity of adult sheep was 58.3%, the mortality was 66.3%, the agent was shown to belong to serotype 16. The disease was brought under control in a short time.

The analysis of the epidemic situation worldwide makes it possible to expect that the most probable route of bluetongue introduction into Russia could be the import of animal carriers; moreover, there were reports from Portugal, Italy, Rumania, Slovakia, Netherlands, Switzerland and Great Britain about cases of imports of bluetongue-positive animals during trade operations with livestock in 2008.

The OIE report for the first half of 2008 informed about 6 bluetongue-affected settlements in 5 regions of Russia (250 bluetongue-positive animals, 2 animals died). All cases were associated with results of laboratory testings of samples from imported pedigree livestock.

Cases of detection of bluetongue-positive animals among imported cattle give no grounds to believe that the given country is bluetongue-affected, as it is necessary to confirm the presence of the disease among native animals. But such animals certainly pose a hazard as they can present a source of infection for transmissible vectors.

In such a situation the termination of animal imports from bluetongue-affected countries could be an optimal decision but it is unacceptable due to the failure of long-term governmental programmes aimed at the increase of efficiency of dairy and meat farming in the country.

Therefore, The Ministry of Agriculture of the Russian Federation developed in 2008 the "Procedure for importation into the Russian Federation from European Union countries of cattle, vaccinated against bluetongue" that was brought to the attention of countries by a special letter.

It is allowed to import in strictly defined subjects of the Russian Federation cattle, vaccinated against serotype 8 bluetongue, from farms with no registered cases of bluetongue, provided they had a bluetongue-negative response by PCR. Moreover, native cattle in Russian regions, surrounding importing farms, should be previously vaccinated against serotype 8 bluetongue.

It is admissible to import also bluetongue non-vaccinated cattle from bluetongue-free farms of exporting countries, provided that animals have no specific antibodies to bluetongue virus.

# POSTER: APPLICATION OF MOLECULAR METHODS FOR DETECTION OF CLOSTRIDIUM BOTULINUM IN ANIMAL FEEDINGSTUFFS

GRENDA, TOMASZ[1]; KWIATEK, KRZYSZTOF[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: Clostridium botulinum, botulism in animals

Inroduction:
Bacteria from the species of Clostridium botulinum are spore – forming, gram – positive, anaerobic rods, which have ability to production the most potent toxin in environment. Botulinum toxins are the etiologic factors of botulism in humans and animals. Historically, differentiation of Clostridium botulinum strains is based on their ability to production one of the seven botulinum toxins signed from A to G. However, botulism is seldom occurring disease but outbreaks of botulism are difficult to control. Cattle and birds are the most affected species. Generally botulism is occurring after ingestion feedingstuff contaminated by botulinum toxin or C. botulinum spores. The aim of this work was application of molecular methods based on PCR and Real – time PCR for detection and determining toxin types of Clostridium botulinum strains from animal feedingstuffs.

Material and methods
The Clostridium botulinum strains: NCTC 887, NCTC 3815, NCTC 8266, NCTC 10281, and own isolates were used for contamination of animal feedingstuffs, and optimization PCR and Real – time PCR. Samples of contaminated feedingstuffs were cultivated under anaerobic conditions in TPGY broth for 48 hours. After incubation, one milliliter of broth was removed to the DNA extraction according to the protocol supplied with commercial kit for DNA extraction.
For examination of prepared DNA screening method based on detection genes which determinate production of non – hemaglutinin component of botulinum toxin (NTHN) was used. Primers for reaction were prepared according to the Raphael, 2007 (Raphael et al., 2007). Real – time PCR was carried out in Roche 2.0 termocycler.
After Real – time PCR, DNA from positive samples were analyzed for determining toxin types: A, B, E, F. For examination multiplex PCR method were used. Primers were prepared according to the CEN, 2008 (CEN, 2008). This method is based on detection of genes which determining production active component of botulinum toxins (BoNT).
DNA from positive samples were also analyzed for determining toxin types C and D. For examination monoplex PCR were used. Primers were prepared according to the Takeshi et al., 1996 (Takeshi et al., 1996). This method is based on detection of genes which determining production of light chain (Lc) of active component of botulinum toxins (BoNT). Monoplex and multiplex PCR analyzes were carried out in Biometra termocycler.

Results and Summary
Every applied molecular methods based on PCR for detection of Clostridium botulinum gave positive results for every contaminated samples of animal feedingstuffs. These methods could be applied for direct, sensitive and fast examination samples which are suspected of contamination by Clostridium botulinum and fast examination samples of fedingstuffs from botulism outbreaks.

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# POSTER: DETERMINATION OF AVIAN INFLUENZA A VIRUSES IN SOME AVIAN SPECIES IN VAN LAKE BASIN BY RT- PCR, THEIR ISOLATION AND SUB-TYPING (THIRD PRESENTING OF DATA)*

BOYNUKARA, BANUR[1]; AKSAKAL, ABDULBAKI[1]; GÜLHAN, TIMUR[1]; İLHAN, ZIYA[1]; EKİN, İSMAIL HAKKI[1]; ADIZEL, ÖZDEMIR[2]; ÖĞÜN, ERDAL[2]; DURMUŞ, ATILLA[2]; ÇÖVEN, FETHIYE[3]; SOLMAZ, HASAN[1]

YÜZÜNCÜ YIL UNIVERSITY, VETERINARY FACULTY[1]; YÜZÜNCÜ YIL UNIVERSITY, ARTS AND SCIENCES FACULTY[2]; BORNOVA VETERINER KONTROL ARAŞTIRMA ENSTITÜSÜ[3]

Key words: Avian influenza, isolation, RT-PCR, Avian Species, Van Lake Basin

In this study, 1910 feces samples were collected from 47 different bird species (transit migratory, winter visitor, migratory and native birds) in Lake Van Basin and the materials were tested by RT-PCR for the detection of avian influenza A virus M2 protein. For this purpose, fresh feces samples were collected using sterile cotton swaps and subdivided into two plastic tubes including transport medium with antibiotics. These samples were transferred in shortest possible time to Yüzüncü Yıl University, Veterinary Faculty, Department of Microbiology. Samples were kept at -80°C until processing. One series of materials was used in embryonated chicken eggs (ECE) and other in RT-PCR. High pure viral RNA kit (Roche) was used for the extraction of viral RNA from materials and transcriptor first strand cDNA synthesis kit (Roche) used for cDNA synthesis. These kits were applied according to the manufacturers' recommendations. Lightcycler (LC) faststart DNA master hybprobe kit (Roche) used for the detection of M2 protein in cDNA samples. RT-PCR studies were carried out in LC 480 (Roche) instrument.
Of the 1910 feces samples, 51 (2.6%) tested positive for M2 protein. A total of 14 (29.7%) bird species Anas acuta, Anas clypeata, Anas crecca, Anas querquedula, Anas platyrhynchos, Anas penelope, Aythya ferina, Aythya fuligula, Himantopus himantopus, Larus argentatus, Larus ridibundus, Phoenicopterus ruber, Tadorna tadorna and Tringa tetanus were detected as M2 protein positive.
The samples tested positive by RT-PCR were inoculated into ECE and 11 (%21.56) influenza A viruses were isolated. Influenza A viruses were isolated from Anas crecca (5), Anas clypeata (4), and Aythya ferina (2) bird species.

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# POSTER: AVIAN INFLUENZA VIRUSES IN WILD BIRDS IN PORTUGAL (2004-2008)

HENRIQUES, ANA MARGARIDA[1]; FAGULHA, TERESA[1]; BARROS, SILVIA[1]; RAMOS, FERNANDA[1]; DUARTE, MARGARIDA[1]; LUIS, TIAGO[1]; FEVEREIRO, MIGUEL[1]

NATIONAL LABORATORY OF VETERINARY RESEARCH[1]

Key words: Influenza virus, wild birds, haemaglutinin, neuraminidase

Wild waterfowl and shorebirds are the natural hosts of all known Influenza A viruses (AIV), which can be grouped in low (LPAI) and highly (HPAI) pathogenic. A total of 15544 samples from wild birds were assayed for AIV by matrix gene rRT-PCR from 2004 to 2008. Ninety eight (0,6 %) samples from ducks, gulls, storks, chickens, partridges and flamingos were positive, exhibiting 9 subtypes of HA (H1, H3, H4, H5, H6, H7, H9, H10, H11) and 8 subtypes of NA (N1, N2, N3, N4, N6, N7, N8, N9). The most prevalent subtypes of HA and NA detected were respectively H5 (25.5 %) and N2 (28.6 %). Twenty five H5 and four H7 AIV subtypes were characterized as LPAI. All samples rRT-PCR positive were subjected to virus isolation. Nineteen isolates were obtained, which represents an infection rate of 19.4 %. The majority of positive samples originated from birds sampled in resting or wintering areas in the northwest coast and centre of Portugal. The H5 subtypes detected in this study share a close relationship with other H5 European strains. The HA and NA genes of H7N3 isolate are closely related to strains reported in England (2006) and the Netherlands (2000). The sequence analysis of NS1 and stalk region of the NA gene confirmed the wild bird origin of these two viruses.

# POSTER: BEAK AND FEATHER DISEASE VIRUS IN AFRICAN GREY PARROTS IN PORTUGAL

HENRIQUES, ANA MARGARIDA[1]; FAGULHA, TERESA[1]; DUARTE, MARGARIDA[1]; RAMOS, FERNANDA[1]; BARROS, SILVIA[1]; LUIS, TIAGO[1]; LAPAO, NARCISO[2]; FERNANDES, TERESA [2]; BERNARDINO, RUI[2]; FEVEREIRO, MIGUEL[1]

NATIONAL LABORATORY OF VETERINARY RESEARCH[1]; LISBON ZOO[2]

Key words: BFDV, Circovirus, Parrot, phylogenetic analysis

Beak and Feather Disease Virus (BFDV), a member of the genus Circovirus, was detected in two dead grey parrots in the Lisbon Zoo (BFDV-PT05) and in a small grey parrot's breeder company near Lisbon (BFDV-PT08). The complete nucleotide sequences of these two Portuguese BFDVs were determined and analysed. Phylogenetic analysis based on complete genomes of BFDV-PT05 and BFDV-PT08 and twenty seven other BFDVs members found in other parts of the world revealed the existence of three new subdivisions of already existing genotype lineages lineages. BFDV-PT08 groups with two other BFDVs of grey parrots from different geographic origins and form one of the new proposed sub-genotype lineages, while BFDV-PT05 constitutes a monophyletic sub-genotype lineage. The nucleotide sequence variation between BFDV-PT05 and BFDV-PT08 was calculated to be 8%, and it ranged from 5% to 12% with the twenty seven other BFDVs found worldwide.

# POSTER: DIAGNOSIS OF RABBIT HAEMORRAGIC DISEASE IN RELATION TO THE GENETIC CHARACTERIZATION OF ISOLATES CIRCULATING IN PORTUGAL

DUARTE, MARGARIDA[1]; FAGULHA, TERESA[1]; RAMOS, FERNANDA [1]; C BARROS, SILVIA[1]; HENRIQUES, MARGARIDA[1]; LUIS, TIAGO[1]; CRUZ, BENEDITA[1]; FEVEREIRO, MIGUEL[1]

LABORATÓRIO NACIONAL DE INVESTIGAÇÃO VETERINÁRIA[1]

Key words: RHDV; RT-PCR; VP60 gene; phylogeny; Portugal

Molecular diagnostic methods are extremely useful for an accurate and timely diagnosis.
For this reason an in house ELISA for Rabbit Haemorrhagic Disease antigen detection used in our laboratory from 1989 to 2004, was replaced by RT-PCR. The chosen method (Kok-Mun Tham et al., 1999) amplifies two regions of 340 and 386 bp located respectively upstream and within the VP60 encoding gene.
According to the RHDV sequences presently available in the database, most of them submitted in the last two years, there is nucleotide variation in the VP60 gene in a significant number of RHDV isolates originated from different countries.
To evaluate if the observed variation could affect the detection of Portuguese RHDV isolates by molecular methods, a comparative study was carried out involving four different RT-PCR systems (Kok-Mun Tham et al., 1999; G. Le Gall et al, 2003; K.Matriz et al in 2006). The primers target different regions on the VP60 gene. RNA extracted from samples suspected of RHDV that arrived to our laboratory from Mainland Portugal, Azores and Madeira Islands since 2004 to October 2008 were used.
The study showed a full agreement between the four RT-PCRs used, which indicates that presumable nucleotide variation in the Portuguese isolates is not affecting any of the methods in particular, proving that the RT-PCRs used in our laboratory are suitable for RHDV detection.
To analyse the variability of the VP60 gene, hence the variability of the primers target regions, two primers located upstream and downstream the coding sequence of gene VP60 were used to amplify the complete VP60 gene of RHDV isolates collected in different years. These primers were selected during this study based on the alignment of nucleotide sequences available in the database. The amplicons of about 2100bp were cloned into pCR2.1 vector and sequenced.
We are reporting here the first results on the genetic characterisation of the RHDV isolates circulating in Portugal in the last 4 years.
The sequencing analysis of large number of Portuguese RHDV isolates will allow the tuning of the molecular method.

# POSTER: SERUM ANTIBODY RESPONSES IN HORSES VACCINATED WITH WEST NILE VIRUS VACCINES

CHIANG, YU-WEI [1]; HATHAWAY, DIANE [1]; JENNINGS, NEIL[1]; NG, TERRY[1]; CHANG, GWONG-JEN[2]; CHU, HSIEN-JUE[1]

FORT DODGE ANIMAL HEALTH[1]; CENTRES FOR DISEASE CONTROL AND PREVENTION[2]

Key words: West Nile Virus, Vaccine, Antibody Response

Serum IgG and IgM antibody responses in horses vaccinated with a conventional killed West Nile virus (WNV) vaccine or with a WNV DNA vaccine were evaluated using ELISA. Horses that were vaccinated with two doses of killed WNV vaccine intramuscularly, three weeks apart, developed substantial IgG response 7 days after the second vaccination. An apparent anamnestic response was detected when the horses were challenged with WNV one year after the second vaccination. After receiving two doses of DNA vaccine, only a few horses developed significant serum IgG response. However, anamnestic IgG response was detected in a majority of the horses vaccinated with the DNA vaccine after challenge exposure to WNV. Very few horses were detected with a positive IgM response after vaccination with either the conventional WNV vaccine or the DNA vaccine. Positive IgM response was detected in all unvaccinated control horses (100%) after challenge exposure while only a small percentage of vaccinated horses had a positive IgM response after challenge. In addition, results from a preliminary study indicate that ELISA with a recombinant WNV non-structure protein (NS-1) may provide a means to differentiate between vaccinated and naturally exposed animals.

# POSTER: APPLICATION OF A RETROVIRAL VECTOR CONTAINING THE CAPSID AND 3C PROTEASE CODING REGIONS OF FOOT-AND-MOUTH DISEASE VIRUS FOR THE INDUCTION OF PROTECTIVE RESPONSE IN GUINEA PIGS

LI, JIONG[1]

LANZHOU VETERINARY RESEARCH INSTITUE (LVRI)[1]

Key words: Foot-and-mouth disease virus; Retroviral vector; MDBK cell; Immune response; guinea pig

Foot-and-mouth disease virus (FMDV) is the causative agent of the economically most important animal viral disease world-wide. Although mortality associated with FMD is usually low, the disease decreases livestock productivity and affects international trade of animal products. Conventional FMDV vaccines using entire virions inactivated have effectively controlled and eradicated Foot-and-mouth disease (FMD) epidemics. But a potential danger still accompanies the use of whole virus vaccine. In order to overcome this problem, several approaches have been used in developing alternative vaccines. A lot of vectors have been used in developing FMD vaccine. Retroviral vectors have yet not been tested for their potential as FMD vaccines vector despite their frequent utilization in researching gene expression and gene therapy. To investigate the retroviral vector suitability for developing FMD subunit vaccine, we constructed the retroviral vector containing the capsid and 3C protease coding sequences of FMDV serotype A, named pBABEpuro-P1-2A-3C. A pseudotyped virus was generated by co-transfection of the retroviral vector and vesicular stomatitis virus glycoprotein G (VSV-G) plasmid into the GP2-293 packaging cell line. Madin-Darby bovine kidney (MDBK) cells were infected with the pseudotyped virus and the transducted MDBK cells were selected for puromycin (2.5 ug/ml) resistance. Expression of the FMDV proteins in selected MDBK cells was detected. Subsequently, the lysate of MDBK cells infected by pBABEpuro-P1-2A-3C virus was used as antigen to immunize guinea pigs and evaluated for its ability to induce a humoral response of FMDV in guinea pigs. In addition, the ability to protect guinea pigs against homologous virus challenge was examined. Guinea pigs were given booster vaccination twice and guinea pigs were challenged 22 days after the third vaccination. Control groups included animals immunized with commercial vaccine, lysate of MDBK cells infected by pBABEpuro virus and lysate of uninfected MDBK cells. All guinea pigs immunized with the lysate of MDBK cells infected by pBABEpuro-P1-2A-3C virus developed specific anti-FMDV antibody and neutralizing antibody. These guinea pigs were completely protected from viral challenge. The results demonstrated the potential of the retroviral vector-based subunit vaccine.

# POSTER: INTRADERMAL VACCINATION OF PIGS AGAINST FMDV WITH 1/10 DOSE RESULTS IN COMPARABLE VACCINE EFFICACY AS INTRAMUSCULAR VACCINATION WITH A FULL DOSE

EBLÉ, P.L.[1]; WEERDMEESTER, K.[1]; VAN HEMERT-KLUITENBERG, F.[1]; DEKKER, A.[1]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]

Key words: foot-and-mouth disease, FMDV, pigs, vaccination, intradermal

We investigated whether intradermal (ID) vaccination against foot-and-mouth disease (FMD) of pigs is suitable as an alternative for the usually used intramuscular (IM) route. We compared vaccine efficacy in groups of pigs in which vaccine administration differed with respect to antigen payload of the vaccine, administrated volume and administration route.

When compared with pigs that were IM vaccinated with a full dose vaccine with a standard antigen payload, pigs vaccinated ID with 1/10 dose of the same vaccine were equally protected against clinical disease and subclinical virus shedding. The ID vaccinated pigs were protected against virus shedding at a significant lower VN-titre as compared to IM vaccinated pigs, suggesting that immune responses other than neutralising antibodies also contributed to protection.

We conclude that the ID route might be a good alternative for IM application, as ID application might induce a very efficient immunological response against FMD and, moreover, because the dose required by the ID route is lower compared to the IM route, ID application may reduce the production costs per dose of FMD vaccine markedly.

# POSTER: GENETIC TYPING OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROM VIRUS (PRRSV) FROM SLOVAKIA

JACKOVA, ANNA[1]; NOVACKOVA, MICHAELA[1]; VILCEK, STEFAN[1]

UNIVERSITY OF VETERINARY MEDICINE IN KOSICE[1]

Key words: PRRSV, EU genotyp, phylogenetic analysis

1. Introductions and Objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive - stranded RNA virus that belongs to the Arteriviridae family, order Nidovirales. We recognized two genotypes, European (EU genotype) and North American (US genotype). EU genotype is discriminated to four subtypes (Stadejek et al., 2006). Objectives of the study were the detection and phylogenetic analysis of PRRSV in domestic pigs from Slovakia.

2. Material and Methods

Forty clinical samples (serum, lymphatic node, lung) were obtained from 8 farms in Western Slovakia from animals suspected of PMWS. Nested RT-PCR was used to amplify of ORF5 region according to Oleksiewicz et al. (1998). Purified PCR products were sequenced. Parcial ORF5 sequences (432 nt) were assembled using SeqMan and MegAlign (DNASTAR).

3. Results

We detected PRRSV by nested RT-PCR in twelve samples from four different farms in Western Slovakia. Occurrence of PRRSV was indicated by respiratory problems of pigs and pathologic changes of organs, mostly lung. We found out by phylogenetic analysis that nucleotide sequences fall into three groups. All of them belong to EU genotype, subtype 1, close to Spanish and Czech isolates.

4. Discussion and conclusions

Our preliminary study indicates that of three phylogenetic groups, some PRRSV isolates from Slovakia were clustered into separate branch. This phylogenetic group will require more exact genetic typing. Our future experiments will be focused on phylogenetic analysis of other open reading regions.

5. Acknowledgements

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# POSTER: GUT HEALTH IN THE EADGENE NETWORK; TRANSCRIPTIONAL RESPONSE IN THE JEJUNUM OF PIGS AFTER CHALLENGE WITH ENTERIC PATHOGENS.

HULST, MARCEL[1]; VAN DER MEULEN, JAN[1]; NIEWOLD, THEO[2]; SMITS, MARI[1]

ANIMAL SCIENCES GROUP[1]; KATHOLIEKE UNIVERSITEIT LEUVEN[2]

Key words: EADGENE, Genomics, Gut health, Pigs, Enteric Pathogens.

The gastrointestinal (GI) tract is a complex ecosystem. Interactions between food components, intestinal cells that absorb nutrients, and the permanent bacterial residents of the intestine (microflora) that help to digest food are important for its proper function. Imbalance in this ecosystem may provoke the loss of the GI tract natural barrier function against pathogens and against harmful components in our diet, resulting in malfunction and consequently into illness. We have studied the effect of enteric pathogens on the GI host response using a home made microarray containing a set of probes (cDNA's) that reflects the genes expressed in the pig's intestinal epithelium (Vet Immunol Immunopathol. 2005 May 15;105(3-4):317-29) and the commercial Operon pig 13K oligoarray. With these arrays, gene expression in the small intestine of pigs was monitored in several experimental models, mainly focusing on the early transcriptional response after challenge with enteric viral (rotavirus) and bacterial pathogens. Especially, our Small Intestinal Segment Perfusion (SISP) model proved to be a reliable model to monitor gene expression in the pig's intestine. This SISP model allows us to generate multiple physically separated segments from the pig's intestine and separately expose these segments to different compounds or pathogens for a maximum period of 16 hours. Because up to ten segments can be installed in the pig's small intestine a number of variables can simultaneously be tested and analyzed in a single pig. Thus, measurements in this model are isogenic, that means without natural occurring variation in gene expression between animals. Using this model, we analyzed the early transcriptional response in the jejunum to an enterotoxin producing non-invasive E. coli (ETEC) strain and an invasive Salmonella strain (Mol Immunol. 2007 Feb; 44(6):1316-22) In addition, the early transcriptional response in the jejunum to an enterotoxin producing rotavirus was studied in germ-free piglets, i.e. in the absence of residential microbes (Arch Virol. 2008 July; 153(7): 1311–1322). These experiments detected several clusters of "response genes". Among them were genes which expression is believed to be crucial for the function of the local (innate) immune defense. Differences in transcriptional responses observed between these different pathogens will be presented.

# POSTER: FMD PREVENTION IN RUSSIA

BORISOV, VLADIMIR[1]; KREMENCHUGSKAYA, SVETLANA[1]; RAKHMANOV, ANATOLY[1]

FGI ALL-RUSSIAN RESEARCH INSTITUTE FOR ANIMAL HEALTH[1]

Key words: system of measures, zonal vaccination, seromonitoring

The present-day tense FMD epidemic situation worldwide requires from each country the development and implementation of effective measures for the given disease prevention and control.

The most efficient strategy for FMD prevention and control, developed with due account for long-term national and foreign experience, is successfully implemented in Russia. It includes the implementation of measures aimed at the prevention of FMD introduction into the territory of the country, predictive modeling and epidemic examination of animal farms, systematic vaccination of animals in zones of high risk of FMD introduction and spread, carrying out of sero- and immunomonitoring, maintenance of vaccine and diagnostica banks, reserves of veterinary facilities and disinfecting agents for eradication of possible FMD outbreaks. In case of their occurrence the disease early diagnosis and agent identification, slaughter and disposal of animals on site, quarantine measures and ring vaccination are provided.

Thanks to the implementation of the given strategy the majority of RF Subjects have FMD-favourable situations for long periods of time. During 10 years (1995-2004) only 3 outbreaks among non-immune animals occurred in the territory of our country as a result of FMD type O introduction: among pigs in the Moskovskaya Oblast (1995) and Primorsky Krai (2000) as well as among cattle in the Amurskaya Oblast (2004). All those outbreaks were eradicated in primary foci of infection.

In 2005-2006 the FMD situation in Russia worsened due to the introduction of FMD type Asia-1 in the Amurskaya and Chitinskaya Oblasts, Khabarovsky and Primorsky Krais, in settlements adjacent to the People's Republic of China. In Russia there were 16 FMD outbreaks in 2005 and 2 FMD outbreaks in 2006. The given type FMD was exotic for Russia and the preventive vaccination against it was not conducted. Following FMD occurrence animals were destroyed in FMD-affected settlements (2146 animals were slaughtered and destroyed; among them there were 2,007 cattle, 128 sheep and goats, 11 pigs) alongside with the implementation of quarantine and restriction measures and vaccination.

Following the eradication of two last outbreaks of FMD type Asia-1 in January and February 2006 in the Chitinskaya and Amurskaya Oblasts the Russian Federation is at present a FMD-free country carrying out the regional vaccination of animals.

Due to a risk of FMD introduction from neighboring countries the preventive vaccination of animals by a trivalent vaccine (against A, O, Asia-1) and seromonitoring are conducted along the southern border at the expense of the federal budget according to the Programme for FMD control. In 2007 in 37 RF regions 11,865.7 ths vaccinations were made in cattle; 15,092.5 ths – in sheep and goats; 116.7 ths – in pigs; more than 3.0 ths - in camels. In 2008 the preventive vaccination of animals against FMD continued along the southern border of Russia; 28 mln doses of trivalent vaccine were used for the given purpose.

The FMD seromonitoring in the buffer zone was held in 2007-2008 with the aim of controlling the immune status. In the FGI "ARRIAH" 7,282 sera samples from different animal species, including wild species, were tested in 2007; 11,678 samples were examined in 2008. It was established that the majority of vaccinated animals had high titres of FMD antibodies in those regions and farms where the recommended vaccination protocol was implemented.

# POSTER: CSF SURVEILLANCE IN WILD BOAR IN POLAND

LIPOWSKI, ANDRZEJ[1]; PEJSAK, ZYGMUNT[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: CSF, wild boar, serosurveillance

Classical swine fever (CSF) is a highly contagious viral disease affecting domestic pigs and wild boar of all ages. CSF is included within the single list of diseases notifiable to the OIE. It causes high economic losses n pig production sector due to preventive culling of pigs, restriction in trade of animals in infected areas and compensation to farmers (4). CSF virus circulates among the wild boar population of Central and Eastern Europe but in Weestern European countries has been also described and is still prevalent in limited number of areas (1). The role of the wild boar as a CSF virus reservoir and possibile source of infection for the domestic pigs is well known (3). This finding is the more important as the population density of the wild boar is still increasing (3). As a result this would impede control schemes due to the increased risk of CSF virus transmission from wild boars to domestic pigs, what makes serosurveillance of CSF in wild boar important.

In Poland the last CSF out break has been detected in 1994. Two years later vaccina-tion against CSF has been banned in all pigs in the whole country (2). In the same year (i.e. 1996) the strategy and design of CSF surveillance programme has been accepted by Polish CVO and then implemented into practice. Its main goal was to demonstrate the absence of CSF virus infection both in domestic pig and wild boar populations. Regarding wild boar on the beginning of the above-mentioned programme the main samples to be tested were tonsils and/or lymph nodes. In 1998/1999 half of the samples tested consisted of blood samples and another half – of organ samples (table 1). During next years there was less and less organ samples in favour of blood samples. It was due to re-design of surveillance system with em-phasis on CSF virus antibody detection. Simultaneously organ samples from all animals found dead or shot showing abnormal behaviour were tested for CSF virus detection. Sample size selected for serological testing depends, among others, on wild boar as well as domestic pig population density in a region concerned, history of CSF in that particular region and CSF status of adjacent countries. It is proper to add that based on the mentioned above serosurveil-lance of CSF, late in 2001, Poland was recognized by European Union as a CSF free country. Negative results of the tests performed allow Poland to maintain this status for years and now our country is one of the Member States free of CSF both in wild boar and domestic pig populations.

Table 1. Population of wild boar, hunting bag and number of blood and organ samples tested for CSF in Poland in the years 1996–2008

| Year | Wild boar population | Hunting bag | Number of blood samples tested/ positives | organ samples tested/ positives |
|---|---|---|---|---|
| 1996 | 82 000 | 76 000 | 46/0 | 1503/0 |
| 1997 | 83 900 | 67 000 | 27/0 | 1368/0 |
| 1998 | 86 600 | 66 000 | 1837/0 | 1985/0 |
| 1999 | 102 000 | 81 000 | 1514/0 | 1452/0 |
| 2000 | 118 300 | 92 000 | 4906/0 | 803/0 |
| 2001 | 123 400 | 93 000 | 5415/0 | 540/0 |
| 2002 | 138 100 | 105 000 | 4605/0 | 315/0 |
| 2003 | 163 300 | 130 000 | 4757/0 | 290/0 |
| 2004 | 160 500 | 122 000 | 6219/0 | 20/0 |
| 2005 | 173 500 | 136 000 | 6614/0 | 477/0 |
| 2006 | 177 100 | 138 000 | 6899/0 | 610/0 |
| 2007 | 178 600 | 118 000 | 6819/0 | 335/0 |
| 2008 | 211 800 | 149 000 | 7879/0 | 96/0 |

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# POSTER: CLINICAL FEATURES OF BLUETONGUE (SEROTYPE 8) IN FRENCH RUMINANTS IN 2007-2008

BELBIS, GUILLAUME[1]; ZIENTARA, STEPHAN[2]; BREARD, EMMANUEL[2]; MAYER, ALAIN[3]; MERCIER, JEAN-LUC[3]; MILLEMANN, YVES[1]

ECOLE NATIONALE VÉTÉRINAIRE D'ALFORT[1]; UMR VIROLOGIE ECOLE NATIONALE VETERINAIRE D'ALFORT[2]; CLINIQUE VETERINAIRE DES 5 VALLEES[3]

Key words: bluetongue, ruminants, serotype 8, France

INTRODUCTION

Since the beginning of the epizootics of bluetongue due to serotype 8 in Northern and Western Europe, the disease has an original host spectrum and affects not only sheep and goats but also cattle. Clinical signs are very polymorph and the severity of the disease varies a lot between herds.

1. MATERIAL AND METHODS

Clinical signs have been observed during ambulatory clinics of Alfort National Vet School in the region of Vouziers (French Ardennes) from September 2007 to February 2008. Cases were defined on the basis of virology (RT-PCR) or serology, as recommended by the French administration (Bexiga et al., 2007).

In parallel, ten Ille de France sheep and eight Saanen goats have been inoculated with a BTV8 strain, and maintained in an A3 animal facility. The clinical score was measured during 39 days post infection (Table 1).

2. RESULTS

2.1. OBSERVATIONS ON OUTBREAKS

The clinical expression of bluetongue due to BTV8 was very varied in cattle, at individual level as well as at herd level.

The clinical expression of bluetongue due to BTV8 in sheep was equivalent to bluetongue due to other serotypes..

Table 1: Clinical signs used for establishing clinical scores

| Category | Clinical sign | Score |
|---|---|---|
| Behaviour | Apathic | 1 |
| | Depressed | 2 |
| | Prostrated | 3 |
| Swelling | Face | 1 |
| | Nose | 1 |
| | Intermandibular | 1 |
| | Lips | 1 |
| | Tongue | 1 |
| | Congestion Skin | 4 |
| | Hooves | 4 |
| Locomotion | Lameness | 2 |
| | Stiffness | 2 |
| Respiratory | Light nasal discharge | 1 |
| | Severe nasal discharge, crusts | 2 |
| | Cough | 1 |
| Digestive | Diarrhoea | 1 |
| Others | Conjunctivitis | 1 |
| | Erosions, ulcers, | 1 |
| | Plaintive bleating | 1 |
| | Drooling | 1 |
| | Subcutaneous haemorrhages of the udder | 1 |

Temperature 40-41°C 1
41-42°C 2
over 42°C 4
The most frequently observed clinical signs were conjunctivitis and nasal discharge, followed by ptyalism, lameness with coronitis, ulcers of the udder, swelling of the face; premature calving and abortions were also reported. Some calves exhibited congenital deformities.

2.2. EXPERIMENTAL INFECTIONS
Incubation lasted 4-6 days for goats, and 2-5 days for sheep. The observed clinical signs are presented in table 2. The course of the disease was generally subacute.
The acme of clinical expression took place at days 9-10 in sheep and a bit later in goats. The maximum clinical score was concomitant to the peak of viral load. It was associated to a more frequent hyperthermia in goats.

Table 2. Frequency of clinical signs observed after experimental infection (number of animals).

| Clinical sign | Sheep | Goats |
|---|---|---|
| Apathy or depression | 9 | 3 |
| Swelling | 8 | - |
| Coronitis | 2 | - |
| Lameness or stiffness | 5 | 7 |
| Nasal discharge | 10 | 8 |
| Diarrhoea | - | 1 |
| Conjunctivitis | 10 | 8 |
| Erosions, ulcers | 3 | 3 |
| Drooling, ptyalism | 7 | 2 |
| Subcutaneous haemorrhages of the udder | - | 7 |
| Hyperthermia | 10 | 3 |
| Total number of animals | 10 | 8 |

3. DISCUSSION AND CONCLUSION
Clinical signs observed in French outbreaks are similar to those observed in border countries in 2006 (Bexiga et al., 2007; Elbers et al., 2008). Clinical signs observed in experimentally infected sheep are similar to those described with other BT serotypes (Darpel et al. 2007).

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Elbers AR, Backx A, Meroc E, Gerbier G, Staubach C, Hendrickx G, van der Spek A, Mintiens K. 2008. Prev Vet Med. 87, 21-30.

# POSTER: DETECTION OF ANTIBODIES AGAINST VHSV AND IHNV IN RAINBOW TROUT.

OLESEN, NIELS JORGEN[1]; CASTRIC, JEANETTE[2]

NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[1]; AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[2]

Key words: seroneutralisation, surveillance, aquaculture, VHS, IHN

The diagnosis and surveillances of VHS and IHN have for more than 50 years been based on virus isolation in cell cultures. Serological test have never been implemented fully for disease surveillance in aquaculture and molecular techniques for virus detection still await final implementation. Antibody detection tests have several advantages compared with virus isolation; especially in cases were water temperature is too high for virus isolation and in endemically infected populations without clinical symptoms of disease. However, the disadvantage of serological test is the slow development and uneven distribution where only part of populations develops detectable antibodies. The aim of the present study is to optimise and to finally validate serological techniques for their implementation as international standards.

The following topics were included in the studies:

• Optimization and validation of serum neutralisation test (SNT) and ELISA according to OIE standards including the organisation of two inter-laboratory proficiency tests.

• Assessment of the importance of the virus isolates used.

• Assessment of the antibody response in single and double infected trout

• How to inactivate virus and at the same time maintain antigenic recognition of fish antibodies.

SOP´s have been written based on the optimisations. The trout complement depended SNT have proven to be very specific and sensitive, as long as the right complement and virus source is used. It was thus shown that trout in SNT only recognise very homologous VHSV isolates. ELISA is less specific and some cross reactions between VHS and IHN and false positives were observed.

While SNT demand cell cultures and infective virus ELISA can in principle be done in all diagnostic laboratories. One problem however is that trout antibodies only react with 3D structures of primarily the viral G-proteins. Only weak denaturisation of these surface proteins will lower the binding significantly. For bio safety reasons it is important to use inactivated virus. Gentle treatment with Triton X-100 has proven to be the most suitable method for solubilisation and inactivation.

Trout can be simultaneously infected with VHS and IHN and develop antibodies against the two.

The ring tests showed that uniform and consistent results can be obtained between laboratories. The longer the laboratories have been working with serological techniques the better results. Weak positives are however often regarded as negative due to relatively high variation in sensitivity between laboratories. Serological surveillance will demand collection of at least 10-30 sera per farm and surveillance can only be performed at population level.

# POSTER: EVALUATION OF A RECOMBINANT VP7 PROTEIN OF BLUETONGUE VIRUS SEROTYPE 8 AND THE DEVELOPMENT OF COMPETITIVE ELISA FOR THE DETECTION OF BLUETONGUE SPECIFIC ANTIBODIES

TISSERANT, FLAVIA[1]; BRÉARD, EMMANUEL[2]; BATTEN, CARRIE A.[3]; SAILLEAU, CORINNE[2]; FOURNIER, LAURA[1]; ZIENTARA, STEPHAN[2]; SELLAL, ERIC[1]

LABORATOIRE SERVICE INTERNATIONAL[1]; AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[2]; INSTITUTE FOR ANIMAL HEALTH (IAH)[3]

Key words: bluetongue, diagnostic, ELISA, recombinant protein

Since 1998 incursions of Bluetongue virus (BTV) have occurred across Europe resulting in outbreaks of disease in both cattle and sheep. BTV diagnosis was traditionally based on clinical signs but more recently laboratory diagnosis is based on the detection of BTV specific antibodies and BTV genome, by enzyme-linked immunosorbent assays (ELISA) and Polymerase Chain reaction (PCR) respectively. The detection of BTV antibodies by ELISA is a rapid assay to determine exposure to BTV infection and/or vaccination. In the last ten years several ELISA assays have been developed and the competitive ELISA (c-ELISA), in which a group-specific monoclonal antibody (Mab) to BTV is used to compete with BTV antibodies present in the sample has proved to be the most sensitive and specific assay.

This paper reports the evaluation of the use of a recombinant structural VP7 protein of BTV serotype 8 (BTV-8 VP7) as antigen for the development of a new c-ELISA. A recombinant baculovirus containing the gene coding for VP7 of BTV-8 was constructed by the French National Reference Laboratory (AFSSA Lerpaz, France) and used to infect insect cells, resulting in the expression of BTV-8 VP7 protein. Characterization of non purified VP7 protein was performed by Western blot and using a standard c-ELISA format by direct adsorption to 96-well plates. Due to its antigenic similarity to the native antigen, BTV-8 VP7 recombinant protein was detected by the group-specific Mab 3-17-A3 produced against BTV-1 (Institute for Animal Health, UK). These characteristics suggested the potential for these two reagents to be developed into an ELISA assay to detect BTV specific antobodies.

The BTV-8 VP7 and Mab 3-17-A3 were optimised for use in c-ELISA. This assay was evaluated and compared to the commercial c-ELISA assay of Laboratoire Service International (France) developed in collaboration with VMRD, inc (USA). Different panels of serum were tested in this study: field bovine samples collected in France during 2008, BTV8-inoculated ruminants and vaccinated animals. Initially the sensitivity and specificity of the assays were evaluated on BTV positive and negative field bovine sera and indicated that the new c-ELISA was very sensitive and specific, total agreement was observed between the two assays (correlation of 98.9%). Secondly, this c-ELISA was demonstrasted to be more sensitive at detecting BTV antibodies in (i) BTV experimentally infected animals (ii) healthy BTV-8 vaccinated animals, particularly after one vaccination when the seroconversion is often difficult to detect.

Analysis of BTV-8 VP7 recombinant protein indicated that it could be a suitable diagnostic reagent for use in c-ELISA to identify BTV-specific antibodies in serum. An advantage of recombinant baculovirus expressed proteins is that they can easily be produced and used without additional purification. The use of the c-ELISA assay that has been developed for the detection of BTV specific antibodies will be compared to the conventional method of serum neutralization (SN), these results will be discussed.

# POSTER: NEW VIRAL VECTOR FOR ORAL VACCINATION DERIVED FROM AN ENTERIC ADENOVIRUS

PERRIN, AURÉLIE[1]; GALEA, SANDRA[1]; PONCET, DIDIER[2]; KLONJKOWSKI, BERNARD[1]; RICHARDSON, JENNIFER[1]

AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[1]; UMR CNRS 2472-INRA 1157[2]

Key words: oral vaccination, enteric adenovirus, rotavirus

Adenoviruses are nonenveloped viruses containing a 36 kbp double-stranded DNA genome packaged into an icosahedral capsid. These viruses have been isolated from many species of mammals, birds or amphibians. Viral vectors have been derived from adenoviruses, most commonly from human adenoviruses of serotypes 2 and 5 (Ad2 and Ad5), which both belong to species C. These adenovirus vectors elicit strong humoral and cellular responses after parenteral immunisation, but have proven less immunogenic upon oral administration, presumably owing to obstacles encountered in the gastrointestinal environment. Nevertheless, oral vaccination provides multiple advantages over other routes of administration, including ease of administration and the induction of not only systemic but also mucosal immune responses. It would thus be of considerable interest to develop new viral vectors that naturally target the intestinal epithelium. In contrast to Ad2 and Ad5, which are associated with respiratory pathologies, adenoviruses of species F (serotypes 40 and 41) are associated with diseases of the gastrointestinal tract. They are the second cause of gastroenteritis of viral origin after rotaviruses. It is plausible that these enteric adenoviruses could be used to create effective orally delivered vaccines. The aim of this work is to exploit the distinctive tropism of the enteric adenoviruses to develop an original vector suitable for oral vaccination. To this end, we have begun construction of a new replication-defective vector based on Ad40. Our strategy is based on that described by He et al. (1998) for Ad5, in which plasmids bearing recombinant adenoviral genomes, generated by homologous recombination between a plasmid comprising most of the adenoviral genome and a shuttle plasmid containing the gene of interest, may be selected on the basis of antibiotic resistance. For our purposes, the Dugan isolate of Ad40, obtained from the ATCC, was cultivated on HEK-293 cells. Viral DNA was isolated following a Hirt extraction protocol. An adenoviral plasmid (pAd40Δ) containing almost the entire viral genome, except the E1 region, the left inverted terminal repeat (ITR) and encapsidation signal (ES), was generated by homologous recombination between the genomic Ad40 DNA and a "capture" plasmid containing two segments of the adenoviral genome corresponding to the extremities of the truncated genome. A shuttle plasmid (pShuttleAd40), containing sequences required for eukaryotic gene expression of genes of interest and for homologous recombination with pAd40Δ, has been constructed. The selected genes of interest encode the green fluorescent protein gene (GFP) for analysis of gene transfer, the C fragment of tetanus toxin (TTFC), a potent mucosal antigen for analysis of immunogenicity and the VP6 gene of rotavirus, for assessment of the capacity of Ad40-based vectors to confer protection in an infectious model. These genes have been subcloned into the shuttle vector and transferred into the adenoviral plasmid following a second homologous recombination. At present, recombinant adenoviruses are being generated by transfection of cells lines able to complement the E1 functions of E1-deleted vectors with the recombinant genomes. Recombinant adenovirus Ad40-ΔE1-GFP/VP6 or TTFC will be amplified and titered and characterised as regards expression of their respective transgenes. It is hoped that the Ad40-based vector will prove more suitable for oral vaccination than conventional Ad-based vaccines.

# POSTER: PHYLOGENETIC ANALYSIS OF THE G-GENE SEQUENCE OF THE ISOLATES OF THE POLISH VIRAL SEPTICEMIA VIRUS (VHSV)

REICHERT , MICHAŁ[1]; MATRAS, MAREK [1]; KAHNS, SØREN[2]; ANTYCHOWICZ, JERZY [1]; OLESEN, NIELS JØRGEN[2]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]; EU REFERENCE LABORATORY FOR FISH DISEASES[2]

Key words: Polish isolates, viral septicemia virus

Phylogenetic analysis of 18 Polish VHS G-gene sequences have been performed based on procedure described by K. Einer-Jensen et al. 2004. The analyzed sequences originated from VHSV isolates collected from different farms in Poland where VHS outbreaks were diagnosed. Six of those isolates were obtained from three farms (two isolates from each farm in one year interval), affected with the disease repeatedly in two subsequent years. The alignment results indicate that some of the isolates are identical with others despite distance between farms from which viruses were isolated. Some of the VHSV isolates differed only by few nucleotides based on the comparison of the G-gene sequence. The results point to the possibility that at least some of the virus variants might arise from the same source. Alignment of the 18 Polish VHS G gene sequences with 61 VHS sequences available at the GenBank indicates that all Polish isolates cluster inside of group Ia and that they are closely related to the published Austrian VHS isolate no.:AU-8/95

# POSTER: SPECTRUM OF DYES WAVES AS A SELECTOR IN REAL-TIME PCR USING FLUORESCENCE RESONANCE ENERGY TRANSFER

KOWALCZYK, ANDRZEJ[1]; MARKOWSKA-DANIEL, IWONA[1]; POMORSKA-MOL, MALGORZATA[1]; URBANIAK, KINGA[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: real-time PCR, dyes, FRET

Introduction

Fluorescence Resonance Energy Transfer (FRET) is powerful technique for characterizing distance-dependent interactions between dyes (1). It is useful in the Real-Time PCR called Primer-Probe Energy Transfer (Pri-ProET). In this PCR, the energy is transferred from the dye on primer to the dye on probe. The emittive wave should be detected in a appropriate time - when the probe is hybridizing less then 100Å from the primer. A longer decay lifetime of donors used in FRET suppress a background of autofluorescence, light scattering and excites acceptor fluorophores. Proper selection of dyes in Pri-ProET qPCR is a matter of efficiency in diagnostic data.
The aim of the study was comparison series of overlapping and non-overlapping acceptors utilizing FAM as a donor.

Materials and methods

Primers and probe sequences for Pri-ProET PCR were designed according to the most conservative region in matrix (M) gene of swine influenza virus (SIV). Sequences for alignment were taken from GeneBank in NCBI website and from SIV strains isolated from clinical material in Poland. Forward primer was labeled with FAM as a donor dye. Acceptor dyes for probe labeling with different absorption maximum, like: HEX, TAMRA, Cy3 and Cy5 were selected.
Briefly, the M genes of H1N1 (A/Sw/Bel/1/98) strain was amplified by RT-PCR and the amplification products were cloned into the pCR 2.1 vector using a dual-promoter TOPO TA cloning kit (Invitrogen). The number of RNA copies was calculated. Tenfold dilutions of the RNA transcripts, ranging from 1 to 1010 copies/µl, were prepared. Pri-ProET PCR was analyzed for every pairs of dyes with the same RNA dilutions of RNA transcripts.

Results

In electrophoresis analyzing all used primer-probe sets permitted amplification of the expected fragment size (170bp) in electrophoresis assay. Specific melting curves with temperature around 74°C, were observed only in sets with Cy5, Cy3 and TAMRA dyes. Four different dyes were tested with the same target RNA transcripts dilutions. Using Cy5 probe dye, in terms of M gene copy number, the limit of detection for the H1N1 subtype was 101 gene copies/µl of in vitro transcribed RNA. For variants with TAMRA and Cy3 dyes sensitivity decreased to 103 gene copies/µl. There were no positive results from Pri-ProET using HEX dyes.
Levels of fluorescence energy were 40oodR, 30oodR, 25oodR with using Cy5, TAMRA and Cy3, respectively. No excitation was obtained with HEX labeling probe.

Discussions

Five dyes with different maximum of absorption were selected to analyze efficiency for Pri-ProET as a diagnostic assay to detect viral RNA of SIV. Maximum emission, around 520nm, due to FAM as donor dye was used as excitation energy for acceptor dyes with the absorbtion 535nm, 552nm, 565 nm and 643nm of HEX, Cy3, TAMRA, Cy5, respectively. In this report it is shown that Cy5 is the most suitable acceptor for Pri-ProET assay. Cy5 is only one dye in selected group of acceptors with non-overlapping spectrum to FAM as a donor. When applying the overlap principle, the residual donor emission in the acceptor channel always limits the sensitivity (2). The mechanism of non-overlapping FRET, as more effective, was considered. To receive better efficiency donor dyes with the maximum emission lower than 520 nm should be selected to interact with other presented acceptor dyes like HEX, Cy3 and TAMRA.

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# POSTER: ACTIVE CONTRIBUTION TO A RISK ASSESSMENT ON THE INCURSION OF CRIMEAN-CONGO HAEMORRAGIC FEVER (CCHF) IN EUROPE IN THE FRAME OF EPIZONE PROJECT WP 7.4: TICKS ON HUNTED WILD ANIMALS IN THE EMILIA ROMAGNA AND LOMBARDIA REGIONS (ITALY), PRELIMINARY RESULTS

MAIOLI, GIULIA[1]; BONILAURI, PAOLO[1]; CALZOLARI, MATTIA[1]; LICATA, ELIO[2]; CASINI, CLAUDIO[3]; MERIALDI, GIUSEPPE[1]; SALA, GIOVANNI[1]; DOTTORI, MICHELE[1]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL' EMILIA ROMAGNA (IZSLER)[1]; REGIONE EMILIA ROMAGNA[2]; AZIENDA UNITA' SANITARIA LOCALE DI MODENA[3]

Key words: ticks, CCHF, climate change, risk assessment

Background

CCHF is an haemorragic disease, transmitted mainly by *Hyalomma* ticks species, which is now reported in Turkey and Greece. One of the aims of the European Epizone project WP 7.4 ("Impact of environmental effects on the risk of the occurrence of epizootic diseases in Europe: Identification and prioritisation") for the year 2009 is to attempt to build a qualitative GIS-based model for the impact of climate change on the risk of incursions of Crimean-Congo haemorrhagic fever virus (CCHFV) in livestock in the EU. Among the data necessary to build this model, presence and distribution of ticks on wild animals, especially hares, are required. In Italy data on ticks distribution are often scarce or very locally focused. This work is an attempt to gather suitable data for the model.

Methods

Ixodid ticks were collected from roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and the European brown hare (*Lepus europaeus*)in Emilia Romagna and Lobardia regions. Animals were sampled after being hunter-killed during the hunting season, which is different for every host species. Ticks were removed and identified following taxonomic standard keys and then stored at -20°C for future analyses. Prevalence differences among host species, tick species and the collection period were 2); linear regression analysis was used to tested by Median Test (Pearson evaluate differences in seasonal intensity of tick parasitization.

Results

A total of 1066 ticks (1009 adults and 57 nymphs) were collected from 139 hunted-killed wild animals, in the period August-November 2008, representing five species of Ixodidae: *Ixodes ricinus* (n=937; 87,82%), *Dermacentor marginatus* (n=112; 10,59%), *Rhipicephalus sanguineus* (n=14; 1,31%), *Pholeoixodes hexagonus* (n=2), and *Haemaphisalys concinna* (n=1). Mean tick intensity (mean number of ticks per host) was determined in each host species. Mean t.i. was 5,23 (CI 95%: 3,82-6,65) for roe deer; 6,4 ticks (CI 95%: 4,11-8,69) for wild boar; 13,85 ticks (CI 95%: 8,67-19,03) for hare; 10,31 ticks (CI 95%: 5,48-15,54) for red deer. Mean t.i. of *I. ricinus* results significantly lower in roe deer than in other species ($p<0,01$). Mean t.i. of I. ricinus shows a significantly positive trend correlated to the sampling months (from August to November) ($F(1,88) = 4,56$; $p<0,05$) and apart from October to November the difference in mean t.i. was always significantly 2(3)=19,99; p (<0,01). All *D. marginatus* ticks were collected on wild boars apart for two specimens collected on hare. The intensity of *D. marginatus* on wild boars does not differ between months.

Conclusions

These data shows that *I. ricinus* is the dominant species in roe deer, red deer and hare in Emilia Romagna and Lombardia regions, while *D. marginatus* is strongly associated with wild boar. Mainly adult stages are associated with large mammals. None *Hyalomma* species were found associated with wild mammals, however other ticks genus such as the *Ixodes, Dermacentor, Rhipicephalus* could act as vectors of CCHFV. *H. m. marginatum* is a thermophilic tick species usually occurring in relatively dry and warm regions of southern Europe, and has been reported all over Italy. This data suggests that these regions are currently at low risk of CCHF expansion even if the virus were introduced by tick infected wildlife or other ways. Climate change could anyway contribute to convert this area in a more suitable ecological niche for *Hyalomma* ticks.

# POSTER: GENETIC DIVERSITY AND CLASSIFICATION OF SUBTYPES OF SMALL RUMINANT LENTIVIRUSES FROM POLAND ON THE BASIS OF ENV GENE ANALYSIS

OLECH, MONIKA[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: small ruminanat lentiviruses, cross-species infection, HMA

Small ruminant lentiviruses (SRLVs) comprise Caprine Arthritis-Encephalitis Virus (CAEV) and Maedi Visna Virus (MVV) which belong to the Lentivirus genus of the Retroviridae family and are considered as a natural pathogens of goats and sheep, respectively. According to the recently proposed nomenclature, based on gag/pol genes sequence analysis, SRLVs belong to four phylogenetic groups (A,B,C,D) which are further divided into several subtypes. Some subtypes have been isolated from both sheep and goats, as a results of interspecies transmission. The main purpose of this study was to investigate genetic diversity of SRLVs isolated from three goats and nine sheep from six geographic regions of Poland. PCR-amplified fragments spanning the V1-V2 region of env gene of SRLVs were analysed by heteroduplex mobility assay (HMA) and further cloned and sequenced for phylogenetic studies.

The results showed that SRLV strains can be clustered in five subtypes including two CAEV-like subtypes (B1 and B2), and three MVV-like subtypes (A1 and two new unknown subtypes, formed only by strains isolated from sheep from Poland). In the one flock more than one strain representing subtypes B1or B2 and subtype A were identified, indicating that interspecies transmission between sheep and goats and vice versa can occurs under natural condition.

Moreover, in one goat the case of co-infection with CAEV and MVV-like viruses was reported for the first time. These results confirmed that the HMA can be used for rapid identification and distribution of diversity within SRLVs, as an alternative method to sequencing. This study gives a first information about distribution and heterogeneity of SRLV subtypes on the basis of env gene analysis.

# POSTER: A PRELIMINARY EXPERIENCE ON THE IMPLEMENTATION OF A LABORATORY CONTINGENCY PLAN

DALLA POZZA, MANUELA[1]; CEOLIN, CHIARA[1]; MARANGON, STEFANO[1]

INSTITUTO ZOOPROFILATTICO SPERIMENTALE VENEZIE[1]

Key words: Veterinary emergency - Laboratory contingency planning

Emergency management of animal diseases is required when there are outbreaks of contagious diseases in a country or region that is free of infection. Under these circumstances, animal diseases have the potential to cause serious socio-economic consequences in a given country or area. The prompt identification of an infectious disease is a prerequisite for the appropriate management of such emergency situations. This is, however, useful only if the infrastructure can respond promptly and adequately to the emergency situation, so that all the necessary measures to contain and then progressively eliminate the infection are implemented. Thus, rapid eradication of highly contagious animal diseases depends on the preparedness to respond to the introduction into a disease-free area and on the way this response is implemented from the time of suspicion. In order to adequately and efficiently handle outbreaks of contagious diseases competent veterinary authorities, including veterinary laboratories, have to be well prepared and should have functioning contingency plans available (1). Laboratory contingency planning (LCP) is designed to mitigate the risk of system breakdown and service unavailability in case of a crises. It is a means to ensure that the laboratory is able to operate effectively and without excessive interruption or delay during an emergency situation (2). Furthermore it allows a laboratory to guarantee that the necessary quality standards will also be met in a crises and serves as a reference manual to all the (laboratory) personnel (3). This paper describes the methodology adopted by the Istituto Zooprofilattico Sperimentale delle Venezie in order to prepare a LCP and the preliminary results related to the implementation of procedures to support competent veterinary authority at farm level for the clinical – anatomo-pathological and diagnostic approach in case of an OIE listed disease. A working group was set up in order to line out where the LCP would fit into the framework of legislation, guidelines and national contingency plans already approved for the different major OIE listed diseases and to define the procedure and the activities aimed at developing the LCP. In order to adequately support the activity of the Official veterinarian at farm level in case of suspicion of an infectious disease, a kit with adequate equipment for the laboratory veterinarian to enter the farm was set up. Moreover an instruction manual with relevant information on major OIE listed disease (virus properties, routes of transmission, clinical signs, sampling, packaging and diagnostic procedures and collection of relevant information on the anamnesis', clinical and epidemiological background of the affected farm) was prepared. A Shift for availability of laboratory staff during non working hours was also implemented. Other activities related to personnel recruitment or mobilization, extra – equipment, reagents and consumable supply and to laboratory exercise to test the organization and procedures already implemented are in progress.

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# POSTER: USE OF FHA FROM BORDETELLA BRONCHISEPTICA AS AN ADJUVANT TO IMPROVE DNA VACCINATION IN SMALL AND LARGE ANIMALS

OKAY, SEZER[1]; ARAGON, VIRGINIA [2]; ROSELL, ROSA[2]; PUJOLS, JOAN[2]; OZCENGIZ, GULAY[1]; RODRIGUEZ, FERNANDO[2]

MIDDLE EAST TECHNICAL UNIVERSITY[1]; UNIVERSITAT AUTONOMA DE BARCELONA[2]

Key words: Bordetella bronchiseptica, filamentous hemagglutinin, adjuvant, DNA vaccination

DNA vaccination is one of the most promising strategies to develop recombinant vaccines either alone, or in protocols of prime-boost. However, one of the main criticisms comes from its low immunogenicity in large animal species and/or against some specific antigens.

In this work, we explore a new strategy based on fusing antigens to the Bordetella bronchiseptica fitohemaglutinin (fHA), an adhesion molecule capable to bind to Antigen Presenting Cells (APCs), including from mucosa, from many different animal origins. Upon DNA vaccination in vivo, fused antigens should be carried out to the sites where fHA have their antigen receptors: mainly in Macrophages, dendritic cells and epithelial cells.

Once demonstrated the capability of fHA-GFP fusion proteins to bind to porcine epithelial cells in vitro, we decided to compare the immune responses induce by a DNA vaccine (pCMV-PQ) encoding two African swine fever virus (ASFV) genes expressed in tandem (PQ), with two DNA constructs encoding same genes as fusions with two different domains from the fHA: pCMV-fHAsPQ and pCMV-fHAlPQ.

Preliminary results obtained using non-syngenic (ICR-CD1 Swiss) mice clearly demonstrated the adjuvant efficacy of fHA. The fusion of either one of the fHA domains to PQ enhanced both the antibody and the cellular responses induced in mice.

We are currently extending these studies, on one hand, to pigs, natural host of ASFV and on the other hand to one of the most threatening virus affecting ruminants: Bluetongue virus (BTV).

The selection of these two viruses obey to several reasons: i) both are diseases of obligatory declaration and between the most threatening viruses for animal health, as listed by the OIE and ii) finding and adjuvant capable to work not only in mice but also in two animal species such porcine and ovine and against diseases and provoked by viruses as different as ASFV (double stranded DNA virus) and BTV (double stranded RNA virus) would mean the definitive consolidation of DNA vaccines as an alternative strategy to control animal diseases.

# POSTER: RETARGETING RECOMBINANT ADENOVIRUSES FOR EFFICIENT ORAL VACCINE DELIVERY

SULEMAN, MUHAMMAD[1]; GAVARD , FRANÇOISE [1]; GALEA , SANDRA [1]; VIAROUGE , CYRIL [1]; ELOIT, MARC [1]; BARRY, MICHAEL [2]; CORTHÉSY, BLAISE[3]; TARTOUR, ERIC [4]; KLONJKOWSKI, BERNARD[1]; RICHARDSON, JENNIFER[1]

AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[1]; BAYLOR COLLEGE OF MEDICINE[2]; CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS[3]; ECOLE NATIONALE VÉTÉRINAIRE D'ALFORT[4]

Key words: Retargeting, oral vaccines, adenovirus, dendritic cells

In the prevention of infectious diseases, orally delivered vaccines may be more effective than their parenterally delivered counterparts, as they are capable of eliciting not only systemic but also mucosal immune responses (IR). Moreover, oral vaccination is more practical, less stressful and safer than systemic injection for vaccination of domestic animals, and represents the only means of vaccinating wildlife. Upon oral vaccine delivery, it is probable that the induction of IR is limited both by the extent to which the digestive epithelium is breached and by the efficiency of antigen capture and presentation by dendritic cells (DC). By consequence, the tropism of a vectored vaccine has important implications for vaccine efficacy. In an effort to optimise the tropism of vaccinal vectors, we have adopted a strategy based on recombinant adenoviruses (Ad) designed so as to facilitate retargeting (Parrott et al., Mol. Ther. 2003). In such vectors, one of the viral proteins, the fiber, is metabolically biotinylated subsequent to the insertion of a biotin-acceptor peptide (bap). This modification permits coupling of Ad to almost any ligand of interest, via an avidin bridge, so long as the ligand can be biotinylated. The tropism of such AdF-bap can be modified in an almost limitless fashion, and thus facilitate assessment of the utility of retargeting Ad toward different populations of cells as regards induction of IR.

To this end, the retargeting of AdF-bap towards intestinal epithelial cells (EC) and DC will initially be evaluated in models elaborated in vitro using either biotinylated antibodies directed against receptors present at the apical pole of intestinal EC or at the surface of DC, or biotinylated versions of the natural ligands of these receptors, when these are available. An AdF-bap vector expressing the marker protein luciferase will be used to study how digestive mucosa is breached in polarised monolayers of intestinal EC. An AdF-bap vector expressing green fluorescent protein (GFP) will be used to evaluate the efficacy of gene transfer in DC by flow cytometry. Subsequently, the interactions between Ad retargeted towards selected receptors of the digestive epithelium and DC of the intestine or the draining lymph nodes will be explored in vivo, after oral delivery in mice, and compared with their capacity to elicit local and systemic IR.

New Ad bearing wild type or biotinylated fibers and expressing one of two model antigens, the C fragment of the tetanus toxin (TTFC), a potent mucosal antigen, or chicken ovalbumin (Ova), for which powerful immunological tools are available, have been constructed. Expression of the corresponding antigens has been verified. Currently, ex vivo retargeting studies are underway using DC derived from bone marrow of C57BL/6 mice. Ad expressing GFP or Ova are being used to evaluate the impact of retargeting on gene expression and its functional consequences as regards initiation of IR, respectively. In particular, following transduction of DC by retargeted Ad-bap-ova, the formation of complexes between MHC-I and an immunodominant T-cell epitope of Ova, as well as the subsequent activation of Ova-specific CD8+ and CD4+ T cells, is being evaluated. Retargeting to intestinal DC will then be evaluated using DC isolated from murine intestinal Peyer's patches and mesenteric lymph nodes. Oral vaccination trials in C57BL/6 mice using Ad-Ova and Ad-TTFC are also in progress to evaluate the potential of these new recombinant viruses for generating local and systemic immune responses.

# POSTER: SEROPREVALANCE OF BOVINE FOAMY VIRUS IN CATTLE FROM POLAND IN RELATION TO SHEDDING OF BFV VIA MILK AND SALIVA

MATERNIAK, MAGDALENA[1]; KUZMAK, JACEK[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: bovine foamy virus, seroprevalence, virus detection in milk and saliva

Bovine foamy virus (BFV) infections are highly prevalent in cattle population worldwide. But virus biology, its mode of transmission and pathogenic potential in natural host are largely unknown. The facts of high similarity of BFV to other foamy viruses, including cross-species infections of humans with simian foamy virus and frequent cases of BFV co-infection with other retroviruses, like BLV or BIV, were reasons of this study on bovine foamy virus infections.

In the presented study BFV prevalence in cattle from Poland was estimated and in terms of BFV transmission, attempts were made to detect BFV in blood, milk and saliva samples of seropositive cattle. Newely developed ELISA test with recombinant BFV Gag protein, as antigen, was used for the investigation of BFV prevalence among cattle coming from three distant regions of Poland: Silesia, Great Poland and Pomerania. Out of 3051 serum samples tested, almost 31% showed the presence of specific antibodies against BFV. The rate of seropositive animals was comparable in all regions. However regarding the herds, the percentage of BFV infected cows ranged between 5% and 65%. Virus isolation was performed by co-cultivation of peripheral blood leukocytes, milk cells and saliva, sampled from BFV serologically positive cows, with permissive Cf2Th cells. Virus isolation was successful for 87% PBLs co-cultures, 68% co-cultures with milk cells and for 65% of saliva co-cultures. Additionally, BFV DNA was detected by nested PCR in all PBL samples, in 79% of milk samples and in 26% of saliva samples.

In general, the study showed significant seroprevalence of BFV among cattle in Poland and the high possibility of BFV transmission through milk and saliva of seropositive cows. Furthermore, high prevalence of BFV and the presence of infectious viral particles in milk and saliva of infected cows can suggest zoonotic potential of this virus.

# POSTER: TRANSCRIPTOMIC MICROARRAY ANALYSIS OF BOVINE MACROPHAGES AND DENDRITIC CELLS RESPONDING TO INFECTION WITH BOVINE IMMUNODEFICIENCY VIRUS AND BOVINE FOAMY VIRUS

ROLA, MARZENA[1]; MATERNIAK, MAGDALENA[1]; KUZMAK, JACEK[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: antigen presenting cells, DNA microarray, immune response, BIV, BFV

Dendritic cells and macrophages are professional antigen presenting cells (APCs) initiating immune response to infection due to their capacity for antigen presentation and activation of naive cytotoxic T lymphocytes. Viruses are able to target antigen presenting cells and modulate their functions causing transient or prolonged changes of the immune response. Although the ability of several viruses to impair antiviral T cell responses is known, the mechanism of immunosuppression induction is not clearly understood. The aim of the study was an analysis of transcriptomic pattern of APCs after infection with bovine immunodeficiency virus and bovine foamy virus, which are natural pathogens of cattle, highly prevalent worldwide. Bovine macrophage cell line (BoMac) and bovine dendritic cells were infected in vitro with BIV and BFV using FBL/BIV112 and Cf2Th/BFV100 cells treated with mitomycin C as virus donors. The expression of genes related to host immune response was examined using cattle A-MEXP-495 microarrays (Roslin Institute Edinburg, UK). Significant changes in genes expression were observed regarding infections with both viruses. While BIV infection resulted in down regulation of genes involved mostly in immunity and defense mechanisms, in BFV infection up regulation of those genes was observed. The results obtained for several genes showing the highest changes in expression level were evaluated by qRT-PCR

# POSTER: DEVELOPMENT OF REAL-TIME REVERSE-TRANSCRIPTION PCR ASSAY FOR THE DETECTION OF AVIAN PARAMYXOVIRUS TYPE 1 RNA IN CLINICAL SAMPLES.

FULLER, CHAD[1]; BRODD, LINA[2]; ALDOUS, ELIZABETH[1]

VETERINARY LABORATORIES AGENCY (VLA)[1]; GUY'S HOSPITAL[2]

Key words: APMV-1 detection Real-Time PCR

Newcastle disease virus (NDV), also known as Avian Paramyxovirus type 1 (APMV-1) is a significant and devastating avian pathogen that circulates throughout the world and affects many species of birds. The current detection method relies on virus amplification in embryonated fowls' eggs (EFEs) and then subsequent virus isolation and characterisation (using serological, molecular and/or in vivo methods), which is a reliable but time consuming method. Real-Time PCR based assays have been effectively employed in the diagnosis of other avian viral pathogens, and have significantly shortened the time taken to confirm diagnosis and enabled elevated through-put with the application of platform-based molecular technologies that are, or have the potential to be, automated. In this study, we report the development of a screening assay using Real-Time reverse transcription PCR incorporating hydrolysis probes that can detect virus isolates from all known lineages of APMV-1. The assay targets a 161bp region of the polymerase (L) gene, selected because of the high level of genetic conservation it exhibits, and includes two probes. It has a detection sensitivity for lineage 1 to 5 strains of NDV (Class II isolates) of approximately 10^1 median embryo infectious doses. A total of 350 (76 positive and 274 negative) clinical samples were tested in comparison with virus isolation in EFEs. These data indicate the assay has a diagnostic sensitivity and specificity of 96.0% and 97.4% respectively. It is proposed that this assay could provide a first line screening tool for the detection of AMPV-1 of in clinical samples.

# POSTER: HPAIV INFECTIONS OF POULTRY AND WILD BIRDS IN CENTRAL EUROPE IN 2007: ELUCIDATING POTENTIAL INTRODUCTION WAYS OF H5N1 INTO GERMANY

STARICK, ELKE[1]; FEREIDOUNI, SASAN[1]; HARDER, TIMM[1]; GRUND, CHRISTIAN[1]; STREBELOW, GÜNTHER[1]; METTENLEITER, THOMAS[1]; BEER, MARTIN[1]; SCHULENBURG, BODO[2]; HAASE, MARTIN[3]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]; OXFORD UNIVERSITY[2]; E.-M.-ARNDT-UNIVERSITÄT[3]

Key words: phylogenetic analysis H5N1 Germany

HPAIV H5N1 was detected in Germany for the first time in February 2006 on the Island of Ruegen (Weber et al., 2007), but also spread to other parts of the country affecting mainly aquatic wild birds and a single poultry holding (Globig et al., 2009). In August 2006 a captive black swan in Germany was the last reported H5N1 European case in 2006 (Starick et al., 2008). The virus re-occurred in Europe in three waves in 2007: in January in Russia, Hungary, and the UK; from June to September in Czech Republic, Germany, France, and Russia (ECTAD 2007); and in November and December in Romania, the UK, Poland, and Germany. Both wild birds and the poultry sector were involved in these outbreaks. We investigated by molecular means possible pathways of re-emergence and spread after introduction in Europe. We present a deepened analysis based on full-length sequences of viruses isolated from wild birds (German Federal States Bavaria, Saxony, Saxony-Anhalt and Thuringia), fattening ducks (Bavaria) and backyard poultry (Brandenburg) in Germany in 2007 (Harder et al., 2009). In addition, H5N1 HPAIV isolated in four further neighbouring European countries in 2006 and 2007 were characterised by whole genome sequencing. A database search for most closely related isolates and the inclusion of these sequences into phylogenetic analyses of all eight gene segments gave insight into the correlation between selected Eurasien and African H5N1 HPAIV isolated in 2006 and 2007. The phylogenetic trees distinguish two subgroups of viruses. The first one comprises viruses isolated in Germany, further European and African countries in 2006 and, interestingly, isolates from early 2007 (Russia, Hungary, England). The second subgroup contains viruses from Mongolia, Korea and Tyva (East Siberia) 2006 and also includes viruses from Kuwait, Czech Republic, Germany, France, Krasnodar (Russia, Black Sea region), Romania and Poland 2007. It could be shown that at least two separate introductions of the H5N1 virus into Germany had occurred in 2006 (cluster 2.2, groups A and B) and that the viruses of 2007 represented yet another group C of this subcluster (Starick et al., 2008). The sources of virus introduction and the routes of its spread remained to a large extent unsolved. Neither temporal nor spatial distribution nor host origin of these viruses currently provides clear evidence for preferred ways of introduction into Europe. However, differences became obvious between viruses isolated from poultry and wild birds in 2007, respectively.

# POSTER: DIAGNOSTIC MICROARRAY FOR SUBTYPING AND PATHOTYPING OF AVIAN INFLUENZA VIRUSES

GALL, ASTRID[1]; HOFFMANN, BERND[1]; HARDER, TIMM[1]; GRUND, CHRISTIAN[1]; EHRICHT, RALF[2]; BEER, MARTIN[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]; CLONDIAG GMBH[2]

Key words: Avian influenza virus, microarray, subtype, pathotype, H5N1

Rapid and reliable methods are fundamental for comprehensive characterization of permanently emerging and evolving avian influenza viruses (AIV). Within surveillance programmes or during outbreaks of highly pathogenic avian influenza, inherent limitations of existing molecular tests, e.g. realtime RT-PCR or sequencing, become evident. Although microarrays provide new possibilities due to their highly parallel approach, their use in diagnostic laboratories is still limited by economical and practical factors as well as insufficient sensitivity.

Here we report a ready-to-use microarray system for sub- and pathotyping as well as specific detection of highly pathogenic H5N1/Asia clade 2.2. The utilized ArrayTube™ system (Clondiag) represents a cost-effective platform involving spotted low density microarrays integrated in reaction tubes and signal amplification by enzyme-catalyzed precipitation staining. Hybridization and analysis are fast and easily conducted with standard laboratory equipment complemented by a simple transmission reader. No extensive special expertise or training is required. The possibility to operate the system in a completely automated 96 well microplate-format (ArrayStrip™) enables processing of high sample numbers.

The developed assay combines one-step "pan" hemagglutinin (HA; amplifying the HAo cleavage site) and neuraminidase (NA) RT-PCRs with subsequent analysis of amplicons using two separate ArrayTubes™. Validation with more than 150 samples (reference strains, virus isolates, cloacal swabs from wild and domestic birds) demonstrated the feasibility. Diagnostic and analytical sensitivity are comparable to realtime RT-PCR (10 genome copies/reaction) while simultaneous detection of HA- and NA-subtypes is possible. Specific reaction patterns of array probes can also be used for differentiation of AIV lineages and assignment of new strains.

The novel diagnostic tool is used within routine diagnostics of the OIE and German National Reference Laboratory for Avian Influenza. Facilitating highly sensitive in-depth analysis for the majority of influenza A-positive cloacal swabs within 24 hours, it can enhance diagnostics even for less well-equipped laboratories. Both arrays have been merged into a single array integrated in 8well-strips (ArrayStrip™) which is currently in validation. Assembled into 96well-microplates the assay is especially suitable for high-throughput laboratories.

# POSTER: DESIGN OF AN AFFORDABLE PROTOTYPE DATABASE FOR STORING DATA OF BIOLOGICAL AGENTS AND THEIR SEQUENCES

GREISER-WILKE, IRENE[1]; ZIMMERMANN, BERND[1]; THIAUCOURT, FRANCOIS[2]

UNIVERSITY OF VETERINARY MEDICINE HANNOVER (HVS)[1]; CENTRE DE COOPÉRATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT (CIRAD)[2]

Key words: database; prototype; molecular epidemiology; genetic typing; sequences

Most veterinary diagnostic laboratories are routinely isolating and identifying pathogens. Very often they are also typing them with various tools which allow typing at much more precise level than simply the species level. These tools could also allow the identification of virulence markers (such as antibiotic resistance in bacteria). On the other hand dating and geo-localising the isolates that are typed open new avenues for molecular epidemiology studies that could help understanding trends in epidemics or even allow better emergency preparedness.

However, much of the data gathered up to now is problematic to use. This is because it may be stored in public databases without data concerning year of isolation and geographic location, or precise case history, or even may be flawed by sequencing errors. In addition, storage in local computers under various formats additionally hinders comparisons with new data.

This lead to the idea of creating a prototype "generic" web-based molecular epidemiology database that is easy to customize for the different pathogens. The main objective of this database is to increase collaboration between European laboratories working in the same field, and provide national as well as international institutions a better picture of the circulation of pathogens worldwide. The corresponding work package was implemented in EPIZONE. It was then agreed that open source programs should be used to keep the costs low, and that the database should be simple to customize and to update. In a second step, the prototype database could be supplemented with additional tools allowing retrieving data from GenBank, allowing automatic alignments to be done, and, last but not least, allowing phylogenetic analysis.

A first prototype database is now available. As a system, a Linux server (e.g. Debian or Ubuntu), with an Apache2 webserver and with MySQL installed is needed. For customizing the scripts, skills in SQL, PERL and HTML are necessary. The software package which has been prepared consists of different PERL scripts, and a database configuration script for the basic table containing the biological and epidemiological data of the organisms, the sequence table, and the table with the sequence types (genes or genomic fragments). The data type and the fields in the basic table are configured during setup. Configuration of the scripts depends on the field names and type of data to be stored. In addition, the field names and the types for the output of data and lists can be configured according to individual needs.

The database consists of an administrator and of a user mode: accession rights are controlled by different user names and passwords. The administrator mode allows adding and modifying entries, and gives access to all fields. In the user mode, data can be accessed and downloaded, but only from fields with free access. The epidemiological data can be downloaded as a text file, or in a format that can be imported into a spreadsheet. The sets of selected sequences can be exported in the FASTA-format, and imported into alignment programs.

The next step will be to customize the database, and generate entries to test usability of the prototype. Then, new modules could be programmed and integrated.

# POSTER: "VALIDATION OF COMMERCIAL REAL TIME RT-PCR KITS FOR DETECTION AND GENOTYPING OF BLUETONGUE VIRUS."

SELLAL, ERIC[1]; BOULEY, SANDRINE[1]; AUDEVAL, CHANTAL[1]; SAILLEAU, CORINNE[2]; BRÉARD, EMMANUEL[2]; ZIENTARA, STÉPHAN[2]; BATTEN, CARRIE[3]

LABORATOIRE SERVICE INTERNATIONAL[1]; UMR AFSSA/INRA/ENVA[2]; INSTITUTE FOR ANIMAL HEALTH (IAH)[3]

Key words: Bluetongue ; RT-PCR; real time;

Since 1998, there have been 13 distinct incursions of bluetongue virus (BTV) from seven different BTVserotypes (BTV-1, 2, 4, 6, 8, 9 and 16) resulting in outbreaks of disease throughout Europe. The need for rapid, robust diagnostic assays for the detection of BTV genome in samples from clinically affected and suspect animals was apparent. In response, LSI (Laboratoire Service International) developed a range of real time RT-PCR assays for the detection of BTV.

Initially a BTV group specific assay targeting the BTV genome segment 1(VP1) was developed. Following initial evaluation of sensitivity using a panel of 200-300 BTV positive field samples at the veterinary Laboratory of Nièvre (in Bourgogne-France), this kit was validated by the European Community Reference Laboratory (CRL) at IAH Pirbright to confirm the specificity of the test on a panel of BTV strains representing all 24 BTV serotypes and related Orbiviruses. The real time RT-PCR kit termed TaqVet® BTV "all genotypes" was able to detect all 24 BTV serotypes. The French National Reference Laboratory (LNR FCO) at Afssa Lerpaz of Maison Alfort determined the specificity and analytical sensitivity of the TaqVet® BTV "all genotypes" kit on European strains and French field samples.
The emergence of BTV-8 in Northern Europe in 2006, along with the introduction of BTV-1 in 2007 and BTV-6 in 2008 initiated LSI to develop BTV real time RT-PCR genotyping kits targeting BTV genome segment 2 (VP2) to identify BTV-1, 8 and 6 genotypes. These kits have been proposed in duplex formats (detection of 2 target: specific serotype and Internal Positive Control (IPC)) and in triplex formats (detection of three targets: BTV serogroup (VP1), specific serotype (VP2) and IPC) in one well. Validation was performed as previous for the TaqVet® BTV "all genotypes kit.

The TaqVet® BTV "all genotypes" and BTV genotype specific kits are now commercially available through www.lsivet.com.

- BTVGroup: TaqVet® BTV "all genotypes" FCO, target: VP1-24 genotypes, Dye detection: Fam/Vic
- BTVGroup/BTV8/IPC: TaqVet® Triplex BTV/FCO-All Genotypes & BTV8, target: VP1/VP2/housekeeping gene, Dye detection: Fam/Vic/Cy5
- BTVGroup/BTV1/IPC: TaqVet® Triplex BTV/FCO-All Genotypes & BTV8, target: VP1/VP2/housekeeping gene, Dye detection: Fam/Vic/Cy5
- BTV1/BTV8/IPC: TaqVet® Triplex BTV/FCO-BTV1 & BTV8, target: VP2/VP2/housekeeping gene, Dye detection: Fam/Vic/Cy5
- BTV6/BTV8/IPC: TaqVet® Triplex BTV/FCO-BTV6 & BTV8, target: VP2/VP2/housekeeping gene, Dye detection: Fam/Vic/Cy5
-BTV6/IPC: Kit TaqVet® Duplex BTV6 Genotyping, target:VP2, Dye detection: Fam/Vic
-BTV8/IPC: Kit TaqVet® Duplex BTV8 Genotyping, target:VP2, Dye detection: Fam/Vic
-BTV1/IPC: Kit TaqVet® Duplex BTV1 Genotyping, target:VP2, Dye detection: Fam/Vic

These studies have highlighted the importance of collaboration between "diagnostic kit manufacturers", veterinary laboratories and reference laboratories to ensure the production of effective, sensitive and specific diagnostic tools that can be used to control the spread of BTV.

# POSTER: EVALUATION OF A NEW VACCINATION STRATEGY AGAINST BLUETONGUE BASED ON A GROUP SPECIFIC ANTIGEN (VP7)

BOUET-CARARO, CORALINE[1]; FOURNIER, ANNIE[1]; SZELECHOWSKI, MARION[1]; BRÉARD, EMMANUEL[1]; RUSSO, PIERRE[1]; THIERY, RICHARD[1]; ZIENTARA, STÉPHAN [1]; KLONJKOWSKI, BERNARD[1]

AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[1]

Key words: vaccine bluetongue virus

Bluetongue virus (BTV), an orbivirus of the Reoviridae family, is the cause of a haemorrhagic disease of ruminants, mainly sheep, occasionally cattle and wild ruminants. The BTV is a non-contagious virus transmitted from one animal to another by the bite of insects of the Culicoides genus. Transmission via seminal fluid and via the placenta has also been reported. Presently, 24 different serotypes of the BTV have been identified by serum neutralization tests. These serotypes have low levels of cross protection and mostly generate neutralizing antibodies to the homologous serotype.

Up to recent years, BTV was known to be endemic in tropical and sub tropical regions but the recent emergence of various serotypes (1, 2, 4, 8, 9 and 16) in Europe has led to dramatic economic losses. Bluetongue is an Office International des Epizooties (OIE) listed disease because of its large economic impact resulting from loss of productivity, mortality, restrictions on animal movements and the costs of setting control measures.

Attenuated or inactivated vaccines are currently available but the protection they afford is serotype specific and they do not allow easy differentiation between vaccinated and infected animals (no DIVA vaccines). Thus, other vaccination strategies, such as virus like particles and recombinant vectors, should be developed to generate safe and cross protective DIVA vaccines.

To this end we have constructed recombinant canine adenoviruses (CAV) containing cDNA copies of different genome segments of BTV serotype 2 (BTV2 strain Corsica 2000). Immunodominant serotype cross reactive T-cell determinants are located within the NS1 nonstructural protein and the structural proteins of BTV cores, namely VP3, VP7 (major core proteins) and VP1, VP4 and VP6 (minor proteins). VP7 vectored by a recombinant capripox virus has proved to be effective in protecting sheep against a virulent heterologous BTV challenge (Wade-Evans, 1996). For this reason, we have constructed a replication defective canine adenovirus serotype 2 that express the VP7 of BTV2 (CAV2-ΔE1-VP7). The construction of this recombinant adenovirus and its evaluation in sheep will be presented. Our data shows that although this recombinant viral vaccine was effective in inducing a humoral response against the VP7 protein and reducing the viral load but it did not afford clinical protection against either homologous (BTV2) or heterologous (BTV8) viral challenge.

# POSTER: DEVELOPMENT OF A EMCV-FMDV RECOMBINANT VIRUS AS A MARKER VACCINE AGAINST FOOT AND MOUTH DISEASE

CAROCCI, MARGOT[1]; GUY, MONIQUE[1]; BAKKALI KASSIMI, LABIB[1]

AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[1]

Key words: FMDV, EMCV, marker vaccine, recombinant virus

The Foot-and-mouth disease (FMD) is economically really important; it is the most highly contagious animal disease. The FMD virus, an aphtovirus of the Picornaviridae family, replicates and spreads extremely rapidly. Recent outbreaks in previously FMD-free countries have demonstrated the vulnerability of countries and the necessity to develop strategies that would allow to stop or at least to reduce the spread of the virus. Following the FMD outbreak in UK in 2001, the European Union has modified the FMD control directive and introduced the possibility to practice urgent vaccination during epizooties. Therefore, the application of this directive has limitations: the current vaccine, an inactivated virus preparation does not allow to differentiate easily vaccinated and infected animals, what can be an obstacle for countries to return to a FMD free status.

The aim of this project is to develop a new approach consisting in the production of a recombinant attenuated chimera virus that would be made of FMDV capsid proteins, and the non structural proteins (NSP) of a close related virus, the Encephalomyocarditis virus (EMCV). Such a recombinant virus would allow vaccination, protection and discrimination of infected and vaccinated animals based on the detection of antibodies against EMCV's NSP for vaccinated animals and antibodies against FMDV's NSP for infected animals.

Since the genomic organisation and the capsid structure of FMDV and EMCV are similar, we decided to substitute, in a genomic cDNA of EMCV, the capsid proteins encoding region by the one of a FMDV strand isolated in France in 2001. In order to attenuate EMCV virulence a partial deletion is introduced in the EMCV 2A protein. The recombinant cDNA will be used to produce a recombinant genomic RNA in vitro which will be transfected in BHK-21 cells to produce the chimera FMDV-EMCV virus. Thus, this virus should be non-virulent and able to induce protection in vaccinated animals. So on, it could be used as a marker vaccine during outbreaks.

Therefore, production of this chimera virus could be faced to some obstacles, like the ability of the EMCV's 3C protease to cleave the precursor of FMDV's capsid proteins, the replication of the chimera genome mainly constitute of EMCV sequence and its encapsidation by the FMDV capsid. Complementary studies are done to highlight those fundamental points.

# POSTER: EVALUATION OF A CHITOSAN-BASED MUCOSAL VACCINE AGAINST THE FOOT AND MOUTH DISEASE IN GUINEA PIGS

COKCALISKAN, CAN[1]; ÖZYÖRÜK, FUAT [1]; ALKAN, MUSA [1]; GÜRSOY, R. NESLIHAN [2]; GÜNBEYAZ, MERVE [2]; ARCA, CIGDEM [2]; UZUNLU, ERGÜN [1]; ŞENEL, SEVDA [2]

FOOT AND MOUTH DISEASE INSTITUTE (SAP)[1]; HACETTEPE UNIVERSITY[2]

Key words: fmd, chitosan, vaccine, mucosal immunity

Foot and Mouth Disease is one of the biggest threat of livestock industry worldwide. The causative agent an apthovirus belonging to picornaviridae. Different strategies have been deployed to control of the disease including culling, mass vaccination and control of movement of susceptible animals. Vaccination is gaining importance even in FMD-free countries because of cost of pre-emtive culling and animal welfare considerations. Use of conventional FMD vaccines have some disadvantages. These vaccines can not prevent infection and subsequent persistence. Mucosal vaccination has potential to solve this problem. An effective mucosal vaccine would induce a strong immune response in mucosal surfaces such as upper respiratory tract where the initial replication of FMD virus occurs. There are number of reports showed both systemic and mucosal response by mucosal immunisation against several pathogens. There are only a few reports utilizing mucosal immunization for FMD vaccine.
In this study we used chitosan as adjuvant and antigen carrying system for FMDV inactivated complete virion antigen in Guinea Pigs (Cavia Porcellus). For this purpose, Guinea Pigs were divided into six grups which are consist of 6 months old six Guinea Pigs. Grup I, II, III and IV received 6.25 µg O1 Manisa FMD whole virus inactivated antigen with different molecular weight of chitosans (Protosan 3%, Chitopharm M %1, Chitopharm M %2, Chitofam L%1 respectively) (1/1 volume total 100µl ) intranasally. Grup V received 6.25 µg O1 Manisa FMD virus antigen with PBS (1/1 volume total 100µl) intranasally . Grup VI received same amount of antijen in Freund's complete adjuvant subcuteneously. Grup VII, VIII, and IX consist of 3 animals each, received only protasan 3%, Chitopharm M and Chitoparm L intranasally as negative controls. The immunisation repeated four times weekly intervals. Nasal wash, saliva and blood sera samples were collected same weekly intervals. All samples were tested in indirect sandwich ELISA for FMD virus spesific antibodies. Results showed that intranasally administered FMD antigen- Chitosan formulation and PBS+Ag induced FMD virus specific serum IgG antibody as well as nasal IgA antibody response whereas subcutaneus administration of FMD antigen with Freund's complete adjuvant did not induce specific nasal IgA response. These results emphasised the importance of administration route which is critical for IgA response. It has a potential of developing a novel nasal FMD vaccine for inducing both systemic and local immune response and studies are needed to try these formulation on the target animal species for FMDV.

# POSTER: COMPARATIVE GENOMICS OF MYCOPLASMA GALLISEPTICUM ISOLATES FROM AN EPIZOOTIC IN HOUSE FINCHES

TULMAN, EDAN[1]; SZCZEPANEK, STEVEN[1]; LIAO, XIAOFEN[1]; ZINSKI, JOSEPH[1]; KUTISH, GERALD[1]; GEARY, STEVEN[1]

THE UNIVERSITY OF CONNECTICUT[1]

Key words: Mycoplasma gallisepticum comparative genomics

Since 1994 the poultry pathogen Mycoplasma gallisepticum has emerged in the house finch, a novel wild songbird host, and has spread across North America. While this epizootic has been used to model disease dynamics, little has been done to characterize the molecular changes occurring in the bacterium. We are applying whole genome sequence analysis to multiple house finch-associated M. gallisepticum (HFMG) isolates obtained throughout time and space during the epizootic to better understand HFMG evolution and how it has adapted to a new host. Thus far we have used pyrosequencing on clonal isolates from four samples (Virginia 1994, New York 2001, North Carolina 2006, and California 2006). While the 1994 HFMG index genome is highly co-linear with the previously sequenced poultry M. gallisepticum strain R, it appears to be as distinct as poultry strains (R and F) are from each other, demonstrating similar strain-specific divergence in repetitive variable surface lipoprotein (vlhA) gene regions and in the presence/absence of several loci. Intra-HFMG genome analysis indicates that approximately 100 to 300 non-vlhA differences have resulted, in later isolates relative to the index. Changes affecting coding potential were observed in multiple genes, however, and likely contribute to the significantly reduced virulence of the California 2006 isolate relative to the index isolate. This study provides a unique opportunity to follow the molecular evolution of an emerging pathogen throughout an ongoing epizootic, which will enable us to better understand basic mechanisms of pathogen adaptation in a new host species.

# POSTER: THE EFFECT OF ERYTHROMYCIN IN COMBINATION WITH SULTRIM OR DANOFLOXACIN ALONE IN PREVENTION OF CRD COMPLEX IN BROILER CHICKENS : A COMPARATIVE STUDY

MIRZAAHMADI, SINA[1]; ZAKERI, AFSHIN[2]; ASAADI TEHRANI, GOLNAZ[1]

ISLAMIC AZAD UNIVERSITY, ZANJAN[1]; ISLAMIC AZAD UNIVERSITY, TABRIZ[2]

Key words: erythromycin; danofloxacin; sultrim; CRD complex; broiler chicken

CRD complex is one of the most important diseases in Iran and all over the world. Mortality , reducing of weight gain and increasing of FCR are caused by CRD complex. Previous studies showed that CRD complex was caused by E.coli and mycoplasma gallisepticum and until now several drugs have been used for prevention and control of it. Using of erythromycin 20% in combination with sultrim or Quinolones drugs alone such as danofloxacin 16.7 % are common and effective in prevention of CRD complex in Iran. In this research we purposed the effect of erythromycin+sultrim combination and danofloxacin in prevention of CRD complex in broiler chickens , to get this aim 450 broiler chickens from Cobb 500 parent stock divided in three similar groups(A, B and C) and subdivided to three replicates of 50 chickens in each group . 200 grams erythromycin 20% + 50 cc sultrim in 200 liters of water was used in group A ( experimental group ) from the 12th day to 15th of growth period , at the same time 60 grams danofloxacin 16.7 % in 200 liters of water was used in group B( experimental group). However, the chickens in group C ( control group) did not get any antibiotic. At 42nd day of age gross lesions , CRD complex induced mortality and FCR of control and experimental groups were calculated. The basis of Anova analysis by using combinations of antibiotics mentioned above , mortality in groups A and B were significantly reduced ( $P < 0.05$ ) compared to group C . furthermore reduction of FCR and mortality in group B( $P < 0.05$ ) with comparison to group C were observed. It can be concluded that using these antibiotic combinations can prevent CRD complex in broiler chickens.

# POSTER: EXPRESSION OF E2 GENE OF CSFV IN MAMMALIAN CELLS AND ITS IMPLEMENTATION IN DIVA

BIDOKHTI, MEHDI[1]; LINDE, ANNA-MALIN[1]; MUNIR, MUHAMMAD[1]; WIDÉN, FREDERIK[1]; BELÁK, SÁNDOR [1]; BAULE, CLAUDIA[1]

NATIONAL VETERINARY INSTITUTE (SVA)[1]

Key words: E2 gene, CSFV, DIVA, Pdual-GC vector, BHK-21 cells

Gene expression in mammalian cells is desirable due to the modern post-translational modifications in comparison with bacteria. Even with the defending mechanisms of mammalian cells against inserted nucleic acids; e.g. RNA degradation due to RNAi, to have the final processed shape of viral glycoproteins, their expression in such cells is desirable.

E2 (gp51-54) is the most antigenic envelope glycoprotein of Classical Swine Fever Virus (CSFV). We attempted to express the E2 gene of CSFV in HS-adapted BHK-21 cells. The E2 gene was amplified and ligated in to the Pdual-GC vector which can be transfected in to both bacterial and mammalian cells. The minipreps were prepared using XL1 Blue MRF' super competent cells according to their protocol. To avoid any BVDV contamination, the cells were adapted to the horse serum (HS-adapted). The prepared pcDNAs were transfected chemically in to the HS-adapted BHK-21 cells using different reagents, according to their special protocols. The transfected cells were treated with optimal dose of antibiotic. Cells having pcDNAs could resistant to optimal dose of Geneticin during antibiotic therapy. When all cells in reagent control flasks died, the dose of Geneticin was held stably in the flasks with pcDNA-transfected cells to let the remaining cells grow. After a while star shapes of clonal cells were seen inside the flasks. During the splitting, some 50 µl of resuspended clonal cells was taken to check for inserted gene by 'Cell PCR'. The results showed the presence of the E2 gene of CSFV using PCR. The western blotting test of lysed clonal cells using monoclonal antibodies has also confirmed the modern post-translational modifications of E2 glycoprotein in mammalian cells. We concluded that viral gene expression in normal host cells can be useful to produce viral proteins such as E2 glycoprotein of CSFV which requires undergoing the modern modification to gain its final shape. This final glycosilated shape of E2 then can be used in order to study the protective immune response induced by E2 in more detail. Furthermore, this can also be implemented to differentiate the infected- from vaccinated- animals with the chimeric virus, that is known as DIVA program.

# POSTER: TRACKING DOWN THE UNKNOWN: EMERGENCES2, A WEB-BASED EARLY WARNING SYSTEM FOR THE DETECTION AND IDENTIFICATION OF EMERGING ANIMAL DISEASES IN BELGIUM

HERR, CECILE[1]; BOONE, IDES[1]; DISPAS, MARC[1]

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE (VAR)[1]

Key words: emerging diseases ; early warning system ; monitoring and surveillance system

Emerging and re-emerging diseases are a major concern since world-wide movements of people, animals and animal products broke down many barriers to their spread.
In 2006, the detection and identification of the unexpected Bluetongue virus serotype 8 required several weeks in Belgium. Later on, the disease caused important economic losses due to export limitation and poor production performances. This unpredictable outbreak highlighted the need for efficient monitoring and surveillance programmes as prerequisites for the early detection of disease and the urgent implementation of control measures.

To take up this challenge, the Belgian sanitary Authorities launched the set up of a Monitoring and Surveillance System (MoSS) for the early detection and identification of emerging diseases in animals. The MoSS implementation includes the development of a new web-based tool designed as a focal point between field veterinarians, vet experts and sanitary authorities. A web interface created in close collaboration with the French INRA Animal Epidemiology Research Unit will allow Belgian vet practitioners to report atypical clinical observations in domestic animals. The system focuses not only on emerging diseases, namely new diseases in a specific population in a given area, but also on known diseases showing unusual clinical expression. In addition to a clinical description, information is collected on spatial and temporal occurrence, characteristics of the affected animals and other relevant epidemiological data. A flexible ascending hierarchical clustering method is used to aggregate similar cases based on clinical signs, affected animal categories and spatio-temporal distribution. Data about the relevant detected clusters are directly available to a network of specialists in pathology, disease control and epidemiology for further investigation.

Further improvements of the system include testing of other clustering methods and the use of outbreak detection methods based on the comparison of the number of new records within a time period compared to a known baseline. The close cooperation with the French INRA Animal Epidemiology Research Unit allows the MoSS to be easily extended across the Belgian border to a transboundary collaboration using sound and comparable data between the countries.

This system based on the centralization of available information will be a critical tool aiming at shortening the detection time of any health-related event of importance in domestic animals. It is a first significant step in the preparedness for the 'unexpected'.

# POSTER: SEROLOGICAL RESPONSE OF SWINE AGAINST NONSTRUCTURAL PROTEIN OF PORCINE CIRCOVIRUS 2

TRUNDOVÁ, MÁRIA[1]; LOBOVÁ, DANA[1]; CELER, VLADIMIR[1]

UNIVERSITY OF VETERINARY SCIENCES BRNO[1]

Key words: circovirus, serology, recombinant protein, ORF3

Porcine circovirus 2 (PCV2) is considered to be an important pathogen associated with a number of different syndromes and diseases in pigs. The genome of PCV2 is a single stranded circular DNA molecule encoding three open reading frames (ORFs). The role of ORF3 protein has still to be determined.
The aim of this work was to express recombinant ORF 3 protein and to demonstrate that infected animals produce ORF3 specific antibodies which could be used in serological diagnostics of PCV2 associated diseases.
The ORF 3 gene was PCR amplified and cloned into pENTRY vector and then transferred into pDest17 vector. Expression of recombinant protein in Escherichia coli was induced by IPTG and resulting protein was purified by metalochelating affinity chromatography. Purified ORF 3 protein was applied as an antigen in indirect ELISA test and immunoblot. Serological reactivity of examined sera with ORF3 and ORF2 proteins used as antigens was compared.
Although the concentration of ORF3 specific antibodies was considerably lower than the concentration of ORF2 antibodies, these antibodies were found in naturally infected as well as in vaccinated animals.
This work was supported by the Grant Agency of the Czech Republic project no. 524/09/0673.

# POSTER: EXPRESSION OF RECOMBINANT ORF1 PROTEIN OF PORCINE TT VIRUS

JAROSOVA, VERONIKA[1]; CELER, VLADIMIR[1]

UNIVERSITY OF VETERINARY SCIENCES BRNO[1]

Key words: anellovirus, pig, nucleoprotein, serology

Torque teno virus (TT) is a small non-enveloped virus with circular, single-stranded DNA genome. TT virus belongs to the genus Anellovirus and may infect humans and different animal species.
The genome of porcine TT virus contains three open reading frames. ORF1 is coding for protein of the nucleocapsid which could be used for serological diagnostics of virus infected animals.
The goal of this work was to express ORF 1 protein of genotype 2 of the virus. The ORF 1 gene was PCR amplified and cloned into pENTRY vector (Invitrogen) and then transferred into pDest17 vector. Expression of recombinant protein in Escherichia coli was induced by IPTG and resulting protein was purified by metalochelating affinity chromatography. Purified ORF 1 protein was applied as an antigen in indirect ELISA test.
This work was supported by the Grant agency of Ministery of youth and education project no. ME08108 and Grant Agency of the Czech Republic project no. 524/09/0673.

# POSTER: PREVALENCE OF PORCINE ANELLOVIRUSES IN THE CZECH REPUBLIC

JAROSOVA, VERONIKA[1]; CELER, VLADIMIR[1]

UNIVERSITY OF VETERINARY SCIENCES BRNO[1]

Key words: anellovirus, pig, PCR diagnostics

Torque teno virus (TT) is a small non-enveloped virus with circular, single-stranded DNA genome. TT virus belongs to the genus Anellovirus and may infect humans and different animal species. Two different genotypes have been identified to date in pigs, without apparent link to any specific disease.

The goal of this work was to estimate the prevalence of both genotypes of porcine TT virus in the Czech Republic. Virus detection was performed by nested PCR with specific primers situated in the highly conserved intergennic region of the virus.

The total of 120 serum samples was used for TT virus detection. TT1 genotype was detected in 61 samples, TT2 genotype was found in 62 samples. Both genotypes were simultaneously detected in 42 samples.

No statistically important difference was recorded between different age categories of pigs (newborn, weaned piglets, saws, gilts). Unexpectedly both TT virus genotypes were found in newborn animals which suggest possible transplacental transmission of the virus.

No link was determined between TT virus infection and clinical manifestation of PCV2 associated diseases.

This work was supported by the Grant agency of Ministery of youth and education project no. ME08108 and Grant Agency of the Czech Republic project no. 524/09/0673.

# POSTER: THE STUDY OF PERIPLASMIC AND OUTER MEMBRANE PROTEINS PATTERNS IN SALMONELLA ENTERICA SUBSP. ENTERICA SEROVAR ABORTUSOVIS STRAINS ISOLATED FROM IRAN

TADJBAKHSH, HSAN[1]; ZAHRAEI SALEHI , TAGHI[1]; AYOUBI, HAMID REZA[1]

FACULTY OF VETERINARY MEDICINE[1]

Key words: Salmonella abortus ovis, Periplasmic protein, Outer membrane protein SDS-PAGE, Iran.

Aim of this study was comparison of periplasmic and outer membrane proteins (OMPs) patterns between Salmonella abortus ovis strains isolated from Iran. Outer membrane proteins were prepared by sonication in triton X-100. Peripasmic proteins were extracted by 500 Mm Sucrose osmatic shock. Peripasmic and OMPs extracted proteins were analysed by SDS-PAGE and silver staining. The results showed that there is considerable difference between periplasmic protein profiles of S. abortusovis strains. The outer membrane proteins patterns were uproximatively similar among the strains. The protein profile differences and number of protein bands showed in figure and tables. In fact these variations indicating a certain genotypic distance between bacterial strains that isolated from different geographical area of Iran and may be useful for describing the epidemiology or at least genetic relatedness of Salmonella abortus ovis wild types.

# POSTER: MODELLING THE EFFECTIVENESS AND RISKS OF VACCINATION STRATEGIES TO CONTROL CLASSICAL SWINE FEVER EPIDEMIC

BACKER, JANTIEN[1]; HAGENAARS, THOMAS[1]; VAN ROERMUND, HERMAN[1]; DE JONG, MART[2]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]; WAGENINGEN UNIVERSITY AND RESEARCH CENTRE[2]

Key words: classical swine fever, intervention strategies, emergency vaccination, marker vaccine, epidemic models

In a recent update of the Dutch contingency plan for controlling outbreaks of classical swine fever (CSF), emergency vaccination is preferred to large-scale pre-emptive culling. This policy change raised two questions: can emergency vaccination be as effective as pre-emptive culling, and what are the implications for showing freedom of infection? Here, we integrate quantitative information available on CSF virus transmission and vaccination effects into a stochastic mathematical model that describes the transmission dynamics at the level of animals, farms and livestock areas. This multilevel approach connects individual-level interventions to large-scale effects. Using this model, we compare the performance of five different control strategies applied to hypothetical CSF epidemics in The Netherlands and, for each of these strategies, we study the properties of three different screening scenarios to show freedom of infection. We find that vaccination in a ring of 2 km radius around a detected infection source is as effective as ring culling in a 1 km radius. Feasible screening scenarios, adapted to the use of emergency vaccination, can reduce the enhanced risks of (initially undetected farm outbreaks by targeting vaccinated farms. Altogether, our results suggest that emergency vaccination against CSF can be equally effective and safe as pre-emptive culling.

This work has very recently been published in J. R. Soc. Interface.

# POSTER: DISTRIBUTION AND ABUNDANCE OF CULICOIDES SPP. (DIPTERA: CERATOPOGONIDAE) AND FIRST RECORD OF SEVEN SPECIES IN TUNISIA

SGHAIER, SOUFIEN[1]; HAMMAMI, MOUNIRA[1]; DKHIL, ABDERRAZEK[1]; DELECOLLE, JEAN CLAUDE[2]; HAMMAMI, SALAH[1]

INSTITUT DE LA RECHERCHE VÉTÉRINAIRE DE TUNISIE[1]; INSTITUT PARASITOLOGIE ET PATHOLOGIE TROPICALE[2]

Key words: Culicoides - Bluetongue - Vector - Survey - Tunisia

Bluetongue (BT) is an infectious arthropod-borne viral disease that affects ruminants, mainly sheep. BT virus (BTV) is a double-stranded RNA virus (Reoviridae: Orbivirus), it is transmitted among its vertebrate hosts by certain species of Culicoides biting midges (Diptera: Ceratopogonidae). The distribution of the disease and the intensity of the infection are dependent on the distribution and abundance of the vector. Between 1999 and 2002 BTV serotype 2 epizootics affected Tunisia and more recently, new outbreaks of BT have been reported in 2007 with isolation of BTV serotype 1 was isolated. The Veterinary Research Institute of Tunisia initiated in 2006 BT vector surveillance study in the country to obtain baseline data on vector occurrence. Midges of the genus Culicoides were trapped monthly from July 2006 through June 2008 on fourteen farms with UV-light traps. A total of 11582 Culicoides specimens were collected, comprising 25 species: C. imicola, C. circumscriptus, C. newsteadi, C. punctatus, C. paolae, C. sahariensis, C. cataneii, C. puncticollis, C. kingi, C. seavus, C. jumineri, C. kurensis, C. pseudopallidus, C. marcleti, C. heteroclitus, C. longipennis, C. odiatus, C. maritimus, C. obsoletus, C. submaritimus, C. univitatus, C. subfasciipennis, C. indistinctus, C. santonicus et C. fasciipennis. The last seven were isolated for the first time in Tunisia. C. imicola was the most predominant female species, constituting 24% of the total catch. The seasonal occurrence of these species is described and discussed.

# POSTER: GENETIC CHARACTERIZATION OF CAUCASUS AFRICAN SWINE FEVER VIRUS ISOLATES

CARMINA, GALLARDO[1]; ISMAIL, KALABEKOV[2]; ALEJANDRO, SOLER[1]; ALEKSANDRA , YELSUKOVA[2]; ELENA, MARTÍN[1]; SODNOM , TCYBANOV[3]; DENIS, KOLBASOV[3]; MARISA, ARIAS[1]

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[1]; NATIONAL VETERINARY VIROLOGY MICROBIOLOGY[2]; NATIONAL VETERINARY VIROLOGY MICROBIOLOGY [3]

Key words: ASF, genotyping, Caucasus, p72, p54, TRS

African swine fever (ASF) is caused by a DNA virus, Asfivirus, and sole member of the Asfarviridae family. ASF has a serious socioeconomic impact on people's livelihoods, participation in international commercial trade, and protein-food security. The potential distribution of the infection is transcontinental; it can occur wherever swine are raised, and therefore most countries free of the infection take serious measures to prevent its entry. The disease is endemic in domestic and wild porcine species in most of sub-Saharan Africa and Sardinia (an Italian island in the Mediterranean). Outbreaks of ASF were reported in 2007-2008 in Georgia and spread to neighbouring countries including the autonomous republic of Abkhazia, Armenia, Russia and Azerbaijan and are likely to spread further to Eastern Europe or other areas where swine are raised.

DNA fragments amplified from the genome of the isolates from domestic pigs and wild boars collected from active outbreaks in the Caucasus region during 2007 and 2008 were sequenced and compared with other ASF virus (ASFV) isolates to establish the genotype of the viruses. Eight Caucasus ASFV isolates were first genotyped by partial p72-gene and full length p54-gene characterization. All of them were classified into domestic-pig cycle associated p72 and p54 genotype II which includes viruses responsible from early ASF outbreak occurred in Georgia in 2007, and additional isolates obtained from Mozambique, Madagascar and Zambia. To recover higher resolution of virus relationships a sub-typing was performed by analysis of tandem repeat sequences (TRS) within four variable regions inside ASFV genome; B602L gene (CVR) and the intergenic regions between genes E146L-E199L, I73R-I329L and I78R-I215L. As in the case of the p72 and p54 genotyping, the TRS sequences obtained from the Caucasus isolates were identical to those obtained from ASFV isolates belonging to p72 and p54 genotype II. This suggests a single origin for the outbreaks in Caucasus regions and indicates that since the reintroduction of the ASF in Georgia in 2007, ASF has easily spread to other countries in the region. This study constitutes the first detailed assessment of the genetic characterization of ASFV field isolates from active outbreaks in Caucasus countries.

# POSTER: INDUCED APOPTOSIS IN BURSA FABRICIUS AND SPLEEN OF INFECTED CHICKENS BY IBDV WITH USING ELECTRONIC MICROSCOPE

ZAKERI, AFSHIN[1]; MIRZA AHMADI, SINA[2]; FADAEI, MARYAM[3]; AZIZIPOUR, AIDIN[4]

ISLAMIC AZAD UNIVERSITY, TABRIZ[1]; ISLAMIC AZAD UNIVERSITY, ZANJAN[2]; TARBIAT MODARES UNIVERSITY[3]; YRC OF ISLAMIC AZAD UNIVERSITY ARDEBIL BRANCH[4]

Key words: Gumboro, Apoptosis, Bursa Fabricius, Spleen

Destruction of infected lymphocytes with virus and peripheral cells and deplation of lymphocytes in bursa and spleen were caused by necrosis and apoptosis. Studying of apoptosis in lymphocytes of bursa and spleen was purpose of this research. In this study , 50 SPF 28 days olds chickens were divided in to tow groups ( control and experimental) with 25 chickens in each group . The experimental group was infected orally by 106EID50 in 1 ml of IR499 virus ( vvIBDV ) and in control group physiological saline solution was used . AT 4th days post infection , all birds were sacrificed and their bursa of fabricius and spleen were taken out and prepared for EM assay and light microscopic study . By light microscopic study about numeration of apoptotic cells , statistic difference were appeared ( P=0.000) between control and experimental groups .By EM apoptotic cells were appeared by submargination of chromatin of nuclear membrane concurrent chromatin condensation in experimental group but there was not any apoptotic cells in control group Apoptosis was appeared by attachment of virus to IgM+ receptors of LB surface and to enforce of cells to secretion of some cytokines.VP2 and 17KD were major viral proteins induced apoptosis in bursa and spleen in infected chickens. In this study and previous studies were demonstrated that IBDV affected chickens with both of necrosis and apoptosis.

# POSTER: AFRICAN SWINE FEVER VIRUS - INFLUENCING THE IMMUNE RESPONSE TO THE TUNE OF CHEMOKINES?

FISHBOURNE, EMMA[1]; DIXON, LINDA[1]; ABRAMS, CHARLES[1]

INSTITUTE FOR ANIMAL HEALTH (IAH)[1]

Key words: African Swine Fever Virus Chemokines

African swine fever (ASF) is caused by a large double stranded DNA virus (ASFV) capable of inducing a severe haemorrhagic fever in domestic pigs and European wild boar and poses a major threat to European pig farming having recently spread to the Caucasus region. The main target cells for ASFV replication are monocytes and macrophages which have a key role in activating and orchestrating the innate and acquired immune responses of the host, partly through the ordered expression and secretion of chemokines.

Microarray data from studies carried out in our lab with ASFV and a review of gene expression studies carried out for other haemorrhagic viruses and DNA viruses identified chemokines and chemokine receptors which were modulated following virus infection. Viruses that cause haemorrhagic fever in man also primarily affect monocytes and macrophages and ASFV shares genomic similarities with poxviruses which have developed mechanisms to differentially regulate the chemokine network, including production of chemokine receptors, chemokine homologues and the secretion of viral chemokine binding proteins.

Large White, specific pathogen free (SPF) pigs from the protected facilities of AFSSA, Ploufragan France were challenged with non virulent (OURT 88/3) or virulent (Benin 97/1 or Virulent Uganda (1965)) ASFV isolates. Blood samples were collected for chemokine gene transcription studies during ASFV infection and plasma for protein work and chemotaxis assays. Real time PCR is being used for gene transcription analysis; peripheral whole blood was collected at 2 day intervals into PAXgene Blood RNA Tubes (PreAnalytix) and total RNA isolation carried out using the PAXgene Blood RNA Kit (PreAnalytix) which was reverse transcribed using SuperScript III (Invitrogen). The chemokines under investigation primarily focus on those that affect monocytes, macrophages, T cells and natural killer cells which have previously been shown to play a role in immunity to ASFV.

An ASFV gene has already been identified that inhibits host signalling pathways and thus transcriptional activation of host immunomodulatory gene transcription and there are genes of unknown function which may modulate chemokine function. It is thought that ASFV will alter parts of the innate immune system, including expression of chemokines and this may vary depending upon the virulence of the isolate.

# POSTER: SEROPREVALENCE OF VECTOR-BORNE DISEASE IN RUMINANTS (CATTLE, SHEEP) AND EQUIDS (HORSE, DONKEY, MULE) IN MIDDLE BLACKSEA REGION IN TURKEY

ALBAYRAK, HARUN[1]; ÖZAN, EMRE[1]

SAMSUN VETERINARY CONTROL AND RESEARCH INSTITUTE[1]

Key words: Akabane, bluetongue, ephemeral fever, equine infectious anemia, seroprevalence.

In this study, seroprevalance of Bluetongue virus (BTV), Akabane virus in cattle and sheep, Ephemeral fever virus in cattle and Equine infectious anemia virus (EIAV) in equids were investigated in Middle Blacksea Region (Samsun, Ordu, Amasya, Tokat, Sinop). Serum samples randomly collected from 200 cattle, 200 sheep and 223 equids (114 horse, 67 donkey, 42 mule) were tested for the detection of seroprevalance of BTV, Akabane, Ephemeral fever and EIAV. Competetive ELISA (cELISA) tests were used to detect antibodies for BTV and EIAV, while blocking ELISA tests were used to detect antibodies for Akabane and Ephemeral fever viruses.

The seroprevalance of BTV was recorded as 3% (6/200), 11% (22/200) in sheep and cattle, respectively. Additionally, the seroprevalance of Akabane was recorded as 0.5% (1/200) and 22% (44/200) in sheep and cattle, respectively. While no antibody against EIAV was detected in equids, seroprevalence of Ephemeral fever virus infection was found as 13.5% (27/200) in cattle.

# POSTER: DEVELOPMENT OF A PAN-VIRUS DNA CHIP FOR DETECTION OF NEW VIRUSES

LARSEN, LARS ERIK[1]; DUPONT, KITT[1]; BOYE, METTE[1]

TECHNICAL UNIVERSITY OF DENMARK[1]

Key words: DNA chip; virus; diagnostic; emerging infections

Introduction

Most diagnostic methods for detection of virus in animals by e.g., ELISA, PCR or culturing require that there is an established knowledge of known differential diagnostic possibilities. In the case of new evolving pathogens there is therefore a huge diagnostic dilemma, especially if the virus does not grow in cell lines normally established in diagnostic labs.

In the past, such new viruses could be identified only by examine infected material by electron microscopy or by attempts to culture the virus in a wide range of cell culture systems. These systems are often insensitive and unspecific.

The aim of the present project was to develop a pan-virus DNA microchip capable of detecting all known and unknown viruses at the genus level. The intended use of the chip was to try to identify if an unknown virus (agent X) is contributing to the development of PMWS in pigs and secondary to develop a tool to be used in the Danish preparedness program for emerging diseases.

Materials and Methods

The virus-chip was constructed with the aim to detect all known avian and mammalian virus on the genus level. The 1272 single stranded DNA probes (approx. 70'mer) were designed by a group in Taiwan (Chou et al., 2006). The probes covered 27 virus families and 106 virus-genera. The microarray was constructed by spotting the probes on a glass slide.

The chip was tested using RNA or DNA extracted from viruses grown in cell cultures, and from tissues and serum from pigs using commercially available kits. After extraction and eventually cDNA synthesis, the DNA was amplified with random primers as previously described (Wang et al., 2003). The amplified and fluorescence marked DNA was then hybridized to the probes at the glass slide. After a washing step, the signal was detected by the use of a commercial available scanner.

Results

Cell culture samples containing know viruses was tested on the chip. The results showed that the chip could identify the correct virus and by that indicating that the chip works albeit the sensitivity was low.

The initial test of samples from PMWS and healthy pigs revealed a significant signal for several viruses present in both diseased and non-diseased animals. For some of these viruses, the results could not be confirmed by PCR. Thus, there seem to be some unspecific binding of host DNA or RNA which have not yet been solved by optimising the extraction procedure.

Conclusion

A pan-virus chip containing probes for more that 100 virus-genera has been constructed and seems to work very well. Due to unspecific binding of host cell RNA/DNA the extraction and amplification protocol has to be improved before the chip can be used in routine diagnostic work. In addition, there is an ongoing cooperation with other partners within the WP2.4 to design a joint "Epizone chip" by compiling probes from several groups.

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# POSTER: IMPROVING THE POTENCY OF MUCOSAL IMMUNISATION USING LIGAND GRAFTED PLGA MICROSPHERES

BRANDHONNEUR, NOLWENN[1]; CHEVANNE, FRANCOIS[1]; VIÉ , VÉRONIQUE[2]; FRISCH , BENOÎT[3]; HUTET, EVELYNE[4]; CHEVALLIER, SYLVIE[4]; LE CORRE, PASCAL[5]; LE POTIER, MARIE-FRÉDÉRIQUE[4]

UNIVERSITÉ - LABORATOIRE DE PHARMACIE GALÉNIQUE[1]; UNIVERSITÉ- INSTITUT DE PHYSIQUE[2]; INSTITUT GILBERT LAUSTRIAT[3]; AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[4]; UNIVERSITÉ- LABORATOIRE DE PHARMACIE GALÉNIQUE[5]

Key words: mucosal, Microspheres, ligand, phagocytosis, macrophages

Purpose: Mucosal vaccination is an attractive alternative to parenteral vaccination allowing the stimulation of humoral as well as cell-mediated responses and the simultaneous induction of systemic immunity. For an efficient mucosal vaccination using microparticles, it is necessary to maximize their uptake by the cells of interest, i.e. antigen-presenting cells (APCs), since a low level of particle uptake by cells is a limiting factor. In this work, we compared and evaluated the influence of ligand grafting on the rate and intensity of uptake of poly (D,L-lactide-co-glycolide) microspheres by pig alveolar macrophages.

Experimental Methods: Coumarin 6 loaded PLGA microspheres with a mean diameter of 2.5 µm were prepared using spray drying technique. Their surfaces were modified by covalent coupling with different ligands (WGA, mannose-PEG3-NH2 and RGD). Their grafting efficiency was quantified and WGA grafting was characterized by confocal laser scanning microscopy (CLSM) and by Atomic Force Microscopy (AFM). In ex-vivo study, different ratios of surface-modified microspheres (MS) were incubated with macrophages (MA) during 2 hours at 37°C. The uptake was quantified by CLSM.

Results: During the ex-vivo study, surface-modified microparticles exhibited significantly highest uptake by the cells compared to plain microparticles. The uptake of WGA-, Mannose-PEG3-NH2- and RGD-grafted microspheres was saturable and inhibited in presence of free ligand and cytochalasin D and at 4°C suggesting a receptor-dependent uptake mechanism. A mathematical analysis of the relative contribution of specific and non specific uptake showed that it was dependent on the ligand grafted, and on the particle-to-cell ratio.

Conclusion: The current work has shown that surface modification by grafting cell-specific ligands on PLGA particles in the micron range increased their uptake by macrophages. The development of surface modified PLGA MS with specific ligand provides opportunities for targeting vaccine to specific cells or intracellular compartments.

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# POSTER: OCCURRENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN NASAL SWABS FROM POLISH VETERINARIANS

ZMUDZKI, J[1]; EMPEL, J[2]; MARSZALEK, N[2]; NOWAK, A[1]; HRYNIEWICZ, W[2]; PEJSAK, Z[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]; NATIONAL MEDICINES INSTITUTE[2]

Key words: nasal swabs, MRSA, ST398, MLST, pigs

Introduction

The aim of this study was to analyze the carrier state of ST398 MRSA clone among representative group of Polish veterinarians supervising pig farms.

Material and methods

In 10-11 of June 2008, 800 participants of the 14th International Annual Pig Conference in Pulawy, Poland, were asked to take part in the project. Nasal swabs were collected from 213 field veterinarians specializing in swine diseases. The mecA-positive isolates were determined using PCR. The S. aureus strains which gave positive results in PCR were further characterized by PFGE using SmaI restriction enzyme, MLST analysis, spa-typing, agr-typing and SCCmec typing. Additionally, the detection of genes encoding virulence factors, such as enterotoxins (sea-see, seg-sei), exfoliative toxins (eta, etb), tsst, lukS-PV/lukF was performed. On the basis of CLSI and CA-FSM standards a susceptibility testing was performed to the following antimicrobials: tetracycline, daptomycin, fusidic acid, erythromycin and clindamycin.

Results

Five (2.35%) of 213 Polish swine veterinarians were positive for MRSA. Four of them (1.9%) were PFGE nontypeable, had ST398, spa-type t034 (n=1) or t108 (n=3), SCCmec type V, agr-type I and were negative for all the toxins analyzed.One MRSA isolate was typeable by PFGE, had ST 45, spa-type t050, SCCmec IV, agr-typeI and harboured three genes coding for enterotoxins seg, sei, sec. All MRSA isolates ST-398-V were susceptible to daptomycin and fusidic acid but resistant to tetracycline, which is very often used in veterinary practice. Three of them presented the MLSB constitutive phenotype. MRSA ST45-IV was susceptible to all antimicrobials tested.

Discussion

This is the first study concerning nasal colonization with ST 398 "livestock associated" MRSA clone among field veterinarians in Poland. The MRSA ST398 Polish isolates were genotypically similar to the strains observed worldwide (1, 3). It seems that pigs being potential carriers increase the risk of colonization of swine veterinarians with selected strains and these strains are likely to develop clinically significant disease (2, 4). The role of antibiotic use remains uncertain. Most reports show an alarming resistance to tetracyclines which are often used in human medicine. There are still many questions to be explained about MRSA in pigs, its consequences for human health and extent to which antimicrobial use in pig production has contributed to the certain situation in hospital. Further epidemiological analysis should be performed to evaluate the possible role of a human and a pig as a vector in spreading the infection between species. MRSA ST45-IV belong to one of the most prevalent clone observed in Polish hospitals in 2005 (5).

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# POSTER: THE SEROPREVALENCE OF BLUETONGUE VIRUS (BTV) SEROTYPES 4, 9 AND 16 INFECTIONS IN CATTLE IN SOME NORTHEASTERN ANATOLIA PROVINCES OF TURKEY

YILDIRIM, YAKUP[1]; YILMAZ, VOLKAN[1]

UNIVERSITY OF KAFKAS[1]

Key words: Bluetongue virus serotypes 4, 9 and 16, cattle, seroprevalence

The present study aimed to determine the presence and seroprevalence of Bluetongue virus (BTV) serotypes 4, 9 and 16 infections in cattle in Northeastern Anatolia Region. One hundred and fifty five bovine serum samples were collected from cattle from three provinces of Northeastern Anatolia; Iğdır, Kars and Ardahan and analyzed for detection of antibodies against BTV 4, 9 and 16 serotypes by virus neutralization test. The seroprevalence of BTV 4, 9 and 16 serotypes were determined as 75.48%, 54.19%, 38.70%, respectively. The antibodies against single serotype were 27.09% of the samples while antibodies for two serotypes were 29.03% of the sera. The antibodies against all three of serotypes were detected in 27.74% of the sera.

# POSTER: EVALUATION OF THE QIASYMPHONY SP FULLY AUTOMATED SAMPLE PREPARATION SYSTEM FOR NUCLEIC ACID PURIFICATION IN VETERINARY DIAGNOSTICS.

FLUEGGE, DENIS[1]; ROTH, LILLIAN[1]; LEIFHOLZ, SANDY[1]; TAMPAKIS, KONSTANTINOS[1]; SCHERER, MARIO[1]

QIAGEN GMBH[1]

Key words: sample preparation, nucleic acid isolation, automation, veterinary pathogen detection, QIAGEN

Real-time PCR techniques have lead to greater work efficiency in nucleic acid (NA)-based analysis of veterinary pathogens. Premixed and validated real-time PCR reagents are commercially available further decreasing hands-on time and error risk. NA extraction now represents the most critical and labor-intensive step in NA-based diagnostic assays. The yield and purity of the isolated NA essentially influence overall sensitivity and robustness of downstream assays.

In veterinary diagnostics, a wide range of pathogens and specimen types creates a demand for flexible and standardized sample processing. In an ideal case, the extraction procedure should provide high yields of pure NA from different pathogens and from diverse types of sample material.

Veterinary laboratories increasingly employ automated procedures for sample preparation to increase efficiency, reproducibility, and process safety of NA extraction. The QIASymphony SP is a novel fully automated system for convenient purification of NA or proteins based on silica-coated magnetic particle technology. Flexible sample capacity from medium- to high-throughput with variable volumes for sample input and elution and a highly versatile application range enable numerous different purification procedures to be performed using a single instrument. Full sample traceability, ready-to-use reagents and hands-off operation from loading of the specimen up to the completed NA extract secure process safety.

The QIAsymphony SP was used for isolation of bacterial DNA and viral NA from a range of veterinary samples. Quantity and quality of the purified nucleic acids were evaluated using spectrophotometric analysis, agarose gel electrophoresis and downstream assays for pathogen detection. The obtained nucleic acids were highly pure and performed well in all real-time PCR tests, demonstrating the absence of inhibitory substances.

# POSTER: PROTECTIVE EFFICACY OF FMDV SUBUNIT VACCINE PRODUCED USING A SILKWORM-BACULOVIRUS EXPRESSION SYSTEM AGAINST TWO CHINESE TYPE ASIA I ISOLATES IN CATTLE

LI, ZHIYONG [1]; YIN, XIANGPING [1]; YI, YONGZHU[2]; ZHANG, ZHIFANG [2]; LIU, JIXING [1]

LANZHOU VETERINARY RESEARCH INSTITUE (LVRI)[1]; BIOTECHNOLOGY RESEARCH INSTITUTE, CAAS[2]

Key words: FMDV, empty capsids vaccine,PD50 test

Cattle vaccinated with a single dose of subunit vaccine containing the capsid and 3C proteinase coding regions of foot-and-mouth disease virus (FMDV) Asia I/HNK/CHA/05 strain were protected when challenged 28 days later with a homologous virus. Here, the 50% bovine protective dose (PD50) test was performed to assess the potency of the subunit vaccine. When challenged with two Chinese isolates, the subunit vaccine could achieve 6.47PD50 (challenged with Asia I/HNK/CHA/05 strain) and 5.20 PD50 (challenged with Asia I/JSL/05 strain) per dose.

# POSTER: REPRODUCTION PORCINE CIRCOVIRUS USING HYBRID CELL CULTURE A4C2

KOLBASOV, DENIS[1]; MALOGOLOVKIN, ALEXANDER[2]; CYBANOV, SODNOM[2]; NADTOCHEY, GRIGORIY[3]

NATIONAL RESEARCH INSTITUTE OF VETERINARY VIROLOGY[1]; NATIONAL RESEARCH INSTITUTE OF VETERINARY VIROLOGY [2]; INSTITUTE OF EXPERIMENTAL VETERINARY MEDICINE[3]

Key words: PCV-1, PCV-2, PCR, cell degeneration, PMWS.

Introduction

There are numerous data about different cell cultures which are sensitive to porcine circovirus 2 type (PCV-2). However, PCV-2 reproduces in vitro without cytopathic effect because it requires using other methods of laboratory diagnostics. The same time Mankertz A. et al., 2006, G. Misinzo et al.,2005 have shown cells degeneration appearance in monocytes cell lines (3D4\31, LD35) infected with PCV-2.

Thus, selection and adaptation cell cultures to PCV-2 within which we have were determined as aim of our investigations.

Materials and methods

In work cells culture PK-15, CV-1, Vero, goat testicles cell culture (GT), piglets testicles cell culture (PT), porcine embryo kidney cell culture (SPEV), SK-6, kidney of Siberian goat cell culture from (PSGK), sublines PSGK-60 and hybrid SPEV and swine splenocyte (A4C2) were used.

Polymerase chain reaction (PCR). Detection and identification porcine circoviruses 1 and 2 types were performed with type-specific primers, which were calculated on base of Cap – genes PCV-1 and PCV-2 fragments. For detection PCV-1 we used conventional PCR with one primers pair limiting 282 base pair (b.p.) on Cap-gene. Identification of PCV-2 was carried out with nested PCR with two primers pair flanking 349 b.p. and 174 b.p. accordingly. Using PCR kit produced by «Syntol» (Russia) polymerase chain reaction PCR were fulfilled.

Virus material.

To infect cells culture we prepared 10% lymph nodes suspension derived from piglets with porcine multisystemic wasting syndrome (PMWS) symptoms. Samples preparation was fulfiled on methods described by Ellis et al., 1999. Cell cultures infection were fulfilled by adsorbtion virus-containing material on semiconfluent monolayer (in 12-16 h. after cell seeding) during 60 min 37°C.

Virus cultivation was carried out in MEM grown medium with 5% fetal serum (HyClone), 5% bovine serum and 2mM L-glutamine.

Results

Using PCR we determined PK-15, SK-6, PSGK, PSGK-60 were contaminated with PCV-1. In cells CV-1, Vero, PT, GT, SPEV, A4C2 circovirus genom wasn't detected.

Taking into account tropism PCV-2 to lymphoid type cells we have selected A4C2 cell line for virus cultivation. Learning of effect virus to cell line was fulfilled within whole cultivation period. On 4th passage 2 days after seeding we observed gradual development of cell alteration. Some monolayer cells refracted light, in perinuclear area of these cells cytoplasm grains we watched. On 4th day appearance round cells, exfoliation ones off glass we observed. During whole cultivation period genome PCV-2 in cell culture was detected by nested PCR.

So, we have found cell lines which are PCV-1 free and suitable for PCV-2 cultivation. Degenerative lesions in infected with PCV-2 cell line A4C2 detailed learning are required and results of it will appear in our further articles.

# POSTER: INTEREST OF SUBUNIT VACCINES AGAINST LOW PATHOGENIC AVIAN INFLUENZA INFECTION IN DUCKS

PREL, ANNE[1]; LE GALL-RECULE, GHISLAINE[1]; NIGNOL, ANNE-CECILE[1]; NIQUEUX, ERIC[1]; AMELOT, MICHEL[1]; JESTIN, VERONIQUE[1]

AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[1]

Key words: Avian Influenza, virus-like particles, duck, H5N3 subtype, low pathogenic

Low-pathogenic (LP) avian influenza viruses (AIV) of the H5 subtype remain an economic threat to commercial poultry worldwide. Domestic ducks can play a pivotal role in the transmission of these viruses to other poultry and different strategies to prevent viral transmission are studied. The combination of recombinant and inactivated vaccines in a prime-boost vaccination has been shown more promising in ducks than inactivated vaccines alone. However, it might be more advantageous to substitute for the boost the inactivated vaccine by a subunit vaccine such as virus-like particles (VLPs), notably regarding the DIVA strategy.

Four years ago, we undertook to develop AI VLPs composed of 3 structural proteins: H5 and N3 derived from a recent French LPAIV, and the matrix protein M1 derived from an Italian H7N1 LPAIV, by using the baculovirus expression system. We generated VLPs that were morphologically identical to the wild-type influenza virus and that expressed antigenic and biologically active H5 and N3 proteins on their surface. We showed that immunisation of specific pathogen free (SPF) Muscovy ducks with purified VLPs induced haemagglutination-inhibiting H5 antibody titres that were considered to be protective according to published data. However, achievement of VLPs composed of a fourth structural protein, the immunogenic matrix protein M2, could be also interesting. Indeed this protein, besides its important role in influenza virus assembly, is well conserved among the different subtypes of AIV and was shown to induce a broader cross-protection in mice following a heterologous viral challenge.

We generated a quadruple recombinant plasmid by cloning the M2 gene derived from the Italian H7N1 LPAIV into the previous triple recombinant plasmid. Quadruple recombinant bacmids were produced and transfected into Sf9 insect cells to generate quadruple recombinant baculoviruses. Electron microscopy examination of Sf9 cell supernatants purified by sucrose density gradient ultracentrifugation revealed the presence of VLPs. SDS-PAGE, western-blot analysis and neuraminidase inhibition assay were performed to check the presence of the four proteins. Haemagglutination and neuraminidase assays confirmed that the HA and NA proteins were biologically active on the surface of the VLPs. A preliminary comparative study of the immune responses induced by the inoculation of the two types of VLP in SPF Muscovy ducks was undertaken. We showed that immunisation with purified quadruple VLPs induced positive haemagglutination-inhibiting H5 and N3 antibody titres for the majority of the animals. However, we did not succeed in demonstrating if the inoculation of these VLPs improved the immune response significantly.

Experimental protection assays with more ducks should be performed to confirm the vaccine interest of VLPs and to check whether the presence of the M2 protein could induce a significant cross-protection. However, achievement of immunogenic H5 LPAIV VLPs should allow the development of safe and effective subunit vaccines to control the spread of these viruses in ducks in a prime-boost strategy. In addition, they could be used for an immunisation by the mucosal route, and perhaps could increase the efficacy of the vaccination.

# POSTER: DIFFERENTIATION OF FIELD AND VACCINE STRAINS OF RABIES VIRUS USING RT-PCR/RFLP

SMRECZAK, MARCIN[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: rabies, oral vaccination. differentiation, RT-PCR/RFLP

Introduction: Rabies is considered as the most dangerous of the notifable zoonotic diseases. After implementation in Poland of the compulsory yearly vaccination of dogs against rabies in 1949, the number of cases of rabies in domestic animals has rapidly decreased. The red fox (Vulpes vulpes) began to replace domestic dogs as the main rabies vector and reservoir. Because of the traditional eradication methods of rabies in red fox were ineffective in 1993 oral vaccination (OV) of wild animals was introduced in Poland. An important element of the monitoring of the OV effectiveness is to differentiate between field and vaccine rabies virus strains isolated from rabies cases on the OV territory. So far strain differentiation was carried using monoclonal antibodies and indirect immunofluorescence technique. However, this method causes difficulties in results interpretation and very often requires previous virus multiplication in cell cultures. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique developed in our laboratory has advantages over other methods (MoAbs) as it is quite easy to perform, relatively fast and gives the results easy for interpretation.

Aim of the study: The purpose of the study was to show the differences between field and vaccine strains of rabies virus using PCR-RFLP method.

Materials and methods: The vaccine strains of rabies virus (SAD Bern and SAD B-19) and rabies virus isolates originating from terrestrial animals, from different regions of Poland, collected by Virology Department (NVRI, Poland) were used in the study. Animal (terrestrial) rabies isolates were derived from animal's brains, diagnosed by means of direct immunofluorescence antibody test. The RNA of the virus, extracted from vaccine strains and positive brain samples were then amplified using/by One-Step RT-PCR followed by heminested RT-PCR. (hnRT-PCR). The total RNA was extracted using the Viral RNA Mini Kit (Quiagen) according to the manufacturer's protocol. For differentiation between field and vaccine strains, amplification of the most conservative fragment of nucleoprotein gene was performed. The restriction enzymes were selected with NebCutter 2.0 software. Restriction fragments were separated by electrophoresis in 2% agarose gels and visualized under the UV light by staining with ethidium bromide.

Results: Amplification of the rabies virus RNA isolated from the samples from terrestrial animals as well as the RNA isolated from the oral rabies vaccines in RT-PCR and hnRT-PCR gave product of 600 bp. Restriction enzymes selected by Neb Cutter 2.0 were checked for effectiveness. There it was chosen one enzyme - Dra I, which gave two restriction fragments of 450 bp and 150 bp for vaccine strains, but didn't cut field strain's amplicons, giving on the gel only one fragment of 600 bp. In the last stage of the study restriction products visualization was done. RT-PCR/RFLP with DraI enzyme is a good, rigid tool for differentiation between street and vaccine strains of rabies virus used for oral vaccination of foxes.

# POSTER: GENETIC CHARACTERISATION OF FOOT-AND-MOUTH DISEASE VIRUS TYPE A CIRCULATING IN TURKEY DURING 1998–2008

PARLAK, ÜNAL[1]; LAURIDS SIIG, CHRISTENSEN[2]; ÖZYÖRÜK, FUAT[1]; AKTAS, SINAN[1]

FOOT AND MOUTH DISEASE INSTITUTE (SAP)[1]; THE NATIONAL FOOD INSTITUTE[2]

Key words: fmd, phylogenetic analysis, Turkey, serotype A

Three genotypes of foot-and-mouth disease virus serotype A were identified as the cause of disease outbreaks in Turkey during 1998–2008. The data presented are concordant with the conclusion that serotype A strains are repeatedly introduced to Turkey from the east and circulate only transiently in farming communities, while type O strains persist and re-emerge from endemic areas of Turkey. The co-circulation of strains belonging to three A genotypes for 10 years, as observed in the present study, is a remarkable difference compared to previous decades in which only one A genotype was transiently circulating, successively being replaced by others.
Sequence information on FMDV type A isolates collected since 1998 to 2008 were gathered by full-length or partial sequencing of VP1 and phylogenetic analysis (Klein et al., 2006; Parlak et al., 2007). The analysis of FMD type A strains revealed the circulation of the genotypes AIran96 (A96), AIran99 (A99) and AIran05 (A05) and a sub lineage of AIran05, AIran05ARD-07, during 1998-2008. A96, A05 and A96 differed from each other by approximately 20% whereas ARD-07 differed from its ancestor, A05, by 5%. A96 and A99 genotype were found to co-circulate during 1999-2004 while A05 and ARD-07 circulated during 2005-2008 and from 2007 and onward, respectively. Due to this rapid evolution and strain replacements, the vaccine strain of choice had to be changed twice with emergence of A05 and ARD-07 causing a significant challenge to diagnosticians and vaccine producers who are supposed to select and produce correct vaccine strain on time (Parlak et al., 2007; Knowles et al., 2008).

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# POSTER: DETECTION OF CAPRINE ARTHRITIS ENCEPHALITIS VIRUS IN THE RUSSIAN FEDERATION

SIDELNIKOV, GEORGE[1]; KOLBASOV, DENIS[1]; BARUISHNICKOVA, ELENA[1]

NATIONAL RESEARCH INSTITUTE OF VETERINARY VIROLOGY[1]

Key words: caprine arthritis encephalitis virus

Today the interest in goat breeding is being increased, so supplements of the domestic caprine population through regular supplies of high-yielding livestock being imported from abroad are in practice. This aggravates the risk of entering a range of goat- and ship-affecting infections including some slow ones like small ruminant lentiviroses (e.g., caprine arthritis encephalitis, visna-maedi), ovine pulmonary adenomatosis.

Caprine arthritis encephalitis (CAE) is an exotic infection in Russia and insufficiently known as yet, thus its virological investigations seem to be timely.

When carrying out these investigations, we first obtained synovial membrane cells of explants taken from newborn colostrum-free goatlings belonging to several breeds like Saanen and some domestic ones.

The obtained cell cultures were estimated for their sensitivity to a CAEV reference strain 75G-63. As a result, the cell culture taken from aboriginal animals appeared less sensitive to the virus.

We isolated CAEV from a Saanen goat begetter that had respiratory affection manifestations using a method of lymphocyte & monocyte co-cultivation together with the obtained cell cultures. The virus proliferation ran in parallel with formation of multinuclear structures in the monolayer. The virus infectious activity was 105 TCID50/cm3. The virus proliferation was confirmed by PCR method. The primers complementary to a CAEV gag-gene sequence and flanking a genome fragment of 394 b.p. in size and also a fragment of 178 p.b. in size in nested PCR, were used.

Subsequently, the both viruses were proliferated in the synovial membrane cell culture. The virus-containing culture liquid was 50 to 100 times concentrated through the dialysis against polyethylene glycol 6000. The prepared concentrate was used as an antigen in agar-gel immunodiffusion test.

Examination of 385 blood serum and colostrum samples showed the antibody were present in 15% samples tested. Thus, the results of our researches show there is some CAE virus circulation among susceptible goat populations in Russia.

# POSTER: OUTBREAKS OF PESTE DES PETITS RUMINANTS IN MOROCCO IN 2008

KWIATEK, OLIVIER[1]; EL HARRAK, MEHDI[2]; DIALLO, ADAMA[3]; ALBINA, EMMANUEL[1]; LIBEAU, GENEVIÈVE[1]

CENTRE DE COOPÉRATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT (CIRAD)[1]; BIOPHARMA[2]; FOOD AND AGRICULTURE ORGANIZATION[3]

Key words: Peste des Petits Ruminants, Morocco

Peste des Petits Ruminants (PPR) is a highly contagious and fatal disease of sheep and goats. It is endemic in sub-Saharan Africa, Middle East and Asia, but until now it has never been described in North Africa except in Egypt. In July 2008, the Moroccan Ministry of Agriculture issued a first report of the disease to the World Organisation for Animal Health (OIE). This notification was based on clinical suspicion and initial laboratory confirmation carried out by BIOPHARMA in Morocco. Within one week, CIRAD, an OIE reference laboratory for PPR, received samples from one of the first outbreaks through BIOPHARMA (Rabat, Morocco), a vaccine production laboratory. Competitive ELISAs either using the virus nucleoprotein (N-ELISA developed by CIRAD) or the haemagglutinin (H-ELISA developed by IAH), were performed to detect the presence of specific antibodies to PPRV. Twenty one out of 24 sera were positive or doubtful in both N and H-ELISA tests. Blood and tissue samples were tested by conventional PCR (Couacy et al., 2002) and by a newly developed real-time PCR based on the Taqman method (unpublished), both tests targeting the N PPR gene. Three out of 10 blood samples and 8 out of 15 tissue samples were found positive by the two tests or at least by real-time PCR assay. Shortly after this first confirmation, 66 new PPR outbreaks were reported between July 20th and August 4th. In August 2008, FAO organised an expert mission in Morocco. At this occasion, a second set of samples was collected and sent to CIRAD. Additional positive results were generated by conventional and real-time methods. From these samples, a virus was isolated on Vero DogSlamtag cells and a PCR Product of 253 nucleotides in length was sequenced for inclusion in the PPR N gene phylogenetic analysis (Kwiatek et al, 2007). Results indicated that the Moroccan PPRV strain was related to other strains of lineage IV such as the Saudi Arabian strain (DQ840195), the Tajikistan strain (DQ840198) and, the more recently identified Chinese strain (EU360596). For further characterisation for the isolate, it is aimed to implement virulence tests and complete the full genome sequence. In order to control the epidemics in Morocco, a large scale campaign of vaccination of sheep and goats began on September 22nd using the attenuated Nigeria 75/1 PPR vaccine strain developed by CIRAD and IAH, 20 years ago. The vaccine is locally produced by BIOPHARMA.

# POSTER: THE INDUCTION OF CELLULAR RESPONSES AGAINST CONSERVED FMDV T-CELL EPITOPES CORRELATES WITH THE LEVEL OF PROTECTION INDUCED BY DNA VACCINES ENCODING FMDV MINIGENES

ARGILAGUET, J.M.[1]; PEREZ-MARTIN, E.[1]; PEREZ-FILGUEIRA, M.[2]; ESCRIBANO, J.M.[2]; SOBRINO, F.[3]; BORREGO, B.[4]; RODRIGUEZ, F.[1]

CENTRE DE RECERCA EN SANITAT ANIMAL[1]; INSTITUTO NACIONAL DE INVESTIGACIÓN Y TECNOLOGÍA AGRARIA Y ALIMENTARIA BIOTECNOLOGIA[2]; CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA[3]; CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[4]

Key words: FMDV, DNA VACCINES, TARGETING ANTIGENS, CELLULAR RESPONSE

Vaccination against FMDV is still controversial, due to the problems associated to classical vaccines based on chemically inactivated virus. Because of its many advantages, DNA vaccination appears as one of the most promising choices, although for large animals the ability to induce strong immune responses must be improved. In this work, we present preliminary experiments showing the protection afforded in swine by a DNA vaccine strategy focused on targeting epitopes to antigen presenting cells (APCs).

We analysed the response induced by immunization with pCMV-APCH I-BTT, a DNA plasmid encoding previously characterised FMDV B- and T-cell epitopes fused to APCH I, a single chain variable fragment (scFv) of an antibody that recognizes the Class II swine leukocyte antigen (SLA II). Immunization of pigs with this construct, even after one single dose, resulted in full protection for 50% of the animals, in which no signs of disease or viral replication were observed, with partial protection for the rest of the pigs receiving the same plasmid. Protection seemed to correlate with the strong induction of specific T-cells prior to FMDV-challenge that secrete IFNg in response to specific stimulation, as well as a rapid development of neutralizing activity after viral challenge.

The fact that this protection was achieved in the absence of anti-FMDV antibodies before viral challenge focuses the attention to the crucial role played by cellular responses in protection against FMD. On the other hand, the possibility of inducing protective cellular responses against highly conserved T-cell epitopes increases the prospect of generating "universal" vaccines against a highly variable virus such as FMDV, one of the main problems currently hindering the development of effective FMDV vaccines.

# POSTER: BLUETONGUE IN DENMARK 2008

RASMUSSEN, LASSE DAM[1]; RASMUSSEN, THOMAS BRUUN[1]; BELSHAM, GRAHAM[1]; STRANDBYGAARD, BERTEL[1]; BOTNER, ANETTE[1]

NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[1]

Key words: Bluetongue, vaccination, ELISA, real time PCR, BTV8

The first case ever of bluetongue (BT) in Denmark was recorded in October 2007, so the biting midge season in spring 2008, was awaited with some anxiety, due to the development of the BT serotype 8 epidemic in the countries south of Denmark. A BT serotype 8 vaccination campaign was planned to take place in 2008 in Denmark, but since no BTV 8 vaccine was available until July 2008, it was not possible to protect the animals before the biting midge season started.

The first outbreak in Denmark 2008 was detected on August 27 in a cattle herd in Bredebro, which is located in the southern part of Denmark, approximately 20 km north of the border with Germany. Initially two animals were suspects for BT based on clinical symptoms so EDTA blood and serum samples were submitted to our laboratory. One of the animals tested positive by ELISA and real time PCR (Shaw et al. J Virol Methods. 2007 145:115-26) (OD% 15,52±1,4, Ct 27,1±0,4).

This herd consisted of 83 cattle (18 bulls, 38 heifers and 27 cows), furthermore there were 13 sheep present. To evaluate the extent of this outbreak it was decided to test the remaining animals in pools of 5 animals. Three pools were found positive for BT virus (BTV) by real time PCR. In each of these pools one animal was positive for BTV. The animals were also tested individually for the presence of antibodies against BTV by ELISA. Of the 75 animals tested 35 were found positive for BTV specific antibodies. The three virus positive cows had an OD% of 4.32±2.97 whereas the 21 antibody positive but virus negative cows had an OD% of 33.03±8.49. The latter results are probably due to the fact that vaccination took place 9 days prior to this measurement and several animals had seroconverted in the intervening days.

The clinical symptoms in the herd were observed in connection with vaccination against BT and the blood submitted for examination was sampled approx. 30 min. after vaccination, which raised the question of whether the positive PCR results could be due to the vaccine. In order to address this issue, a study was performed in which blood samples were collected at short time intervals immediately before vaccination until 96 h after vaccination (sampling times: 0.25, 1, 4, 24, 48 and 96 h). Neither BTV specific antibodies nor PCR product could be detected at any of these times after vaccination.

From the first outbreak, a newly infected calf (still sero-negative) was brought to our animal facilities at Lindholm in order to be able to follow the development of the BTV antibodies and the level of viral RNA in the blood. Frequent blood samples were taken and analyzed for a period of three months after the BT diagnosis. The calf seroconverted one week after bluetongue was diagnosed and subsequently the level of antibodies increased for a month. Real time PCR values remained at an almost constant level throughout the entire three month period, Ct value at diagnosis was 22.3 and at slaughter Ct 24.8. Sequencing of parts of segment 2 showed 100% homology with BTV 8 isolates circulating in Holland in 2006.

In total in Denmark during 2008 some 15 outbreaks were registered. Out of approx. 65 clinical suspicions 11 were found positive and the remaining four outbreaks were discovered by the routine surveillance of bulk milk. All outbreaks were located in the south western part of the country but several outbreaks were north of the original vaccination zone resulting in an extension of the vaccination zone. In 2009 the entire country is included in a mandatory vaccination zone.

# POSTER: PERSISTENCE OF FOOT-AND MOUTH DISEASE VIRUS IN RUMINANTS

STENFELDT, CAROLINA[1]; BELSHAM, GRAHAM J[1]; TJORNEHOJ, KIRSTEN[1]; ALEXANDERSEN, SOREN [2]

NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[1]; NATIONAL CENTRE FOR FOREIGN ANIMAL DISEASE[2]

Key words: FMD, carriers, host-response, qPCR

During the spring of 2008, a new clinical project, with the aim of investigating mechanisms involved in development of FMD carrier animals, has been launched in the new FMD facilities of the Danish Veterinary Institute located at Lindholm Island.
The project is based on a series of animal experiments, investigating the host response to FMD infection in sheep and cattle.

FMD infection in ruminants involves initial viral replication in pharyngeal epithelia, from where the virus spreads systemically via the lymphatic system. Characteristic vesicular lesions develop in the cornified stratified squamous epithelia of the coronary bands and oral cavity within a few days of infection. Viremia occurs within 2-3 days of infection, but is rapidly cleared through the effect of circulating antibodies of the adaptive immune response. The host response involves initial activation of the innate immune response, with activation and recruitment of effector-cells, and subsequent activation of T- and B-cells, leading to the production of circulating antibodies, as well as activation of cytotoxic T-cells.
In ruminants, approximately 50% of animals infected with FMDV develop into persistently infected carrier animals, with intermittent excretion of live virus, whilst remaining animals clear the infection effectively. Previous experiments have indicated that the site of persistent viral replication is located in pharyngeal lymphoid tissue, as well as the basal epithelia of the dorsal soft palate
In these locations, FMDV is capable of persistent replication, without being detected by the host cellular immune response, which would normally be expected to clear virus infected cells.

In an ongoing series of experiments, animals of 4-5 moths of age are infected with FMD O UKG 34/2001, either through subepidermo-lingual injection or direct contact with inoculated animals. Animals are kept for approximately 2 to 4 months, and the progression of infection is monitored through samples of oropharyngeal fluid (probang samples) and serum, which are analysed for presence of live virus and development of antibodies.
During different fixed time points of the infection, biopsy samples of epithelial and lymphoid tissues from the pharynx and dorsal soft palate are collected with the use of an endoscope equipped with biopsy forceps.
Biopsy samples are used to investigate the host's cellular immune response at different time points during the infection, as well as the presence of FMDV antigen using immunohistochemistry. Samples will also be used to investigate expression of genes related to the innate and adaptive immune responses through qPCR at the mRNA level.

# POSTER: FURTHER EVALUATION OF THE DIVA VACCINE PROPERTIES OF THE CHIMERIC PESTIVIRUS CP7-E2GIF USING COMMERCIALLY AVAILABLE CSFV ELISA KIT SYSTEMS

RASMUSSEN, TANYA[1]; BRUUN RASMUSSEN, THOMAS [1]; UTTENTHAL, ÅSE[1]

NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[1]

Key words: CSFV, DIVA vaccines, DIVA diagnostic

Classical swine fever (CSF) is a highly contagious and often fatal viral disease affecting domestic pigs and wild boars worldwide. It is an important disease from both an economic and sanitary view as an outbreak impairs internal and international trade of pigs and pig products and requires the slaughtering of diseased and exposed pigs.
Efficient vaccines against CFSV are available for emergency vaccination or prophylactic use, but they are not recommended to be used as a tool to control outbreaks as they are live attenuated vaccines that elicit the same antibody patterns as those observed in naturally infected animals. Two marker vaccines against CSFV are registered but they are both subunit vaccines based on E2 protein and they are less efficient than the live vaccines. Therefore large efforts have in recent years been put into developing new and safe marker or DIVA (differentiating infected from vaccinated animals) vaccines. To use the DIVA principle the diagnostic tools used needs to be highly specific and at the same time very sensitive.
This presentation evaluates the DIVA potential of the chimeric pestivirus CP7_E2gif, earlier presented as a potential live vaccine candidate towards CSFV (Rasmussen et al., 2007). Preliminary results have shown that E2 specific DIVA detection is an option (Rasmussen et al., 2008). The chimeric pestivirus CP7_E2gif is unique as no CSFV sequences are present in the genome, since it consists of the backbone of BVDV CP7 and the complete envelope protein E2 from BDV strain Gifhorn.

The aim of this study was to further evaluate the reliability of commercially available CSFV antibody ELISA kit systems for their use as DIVA diagnostics for a CP7_E2gif based DIVA vaccine. Seven different CSFV antibody ELISA test kits from four different companies (Prionics, Synbiotics, IDEXX Laboratories and Labour Diagnostic), targeting distinct antibodies (E2, Erns, NS3) of only CSFV or CSFV, BDV and BVDV were used.

Testing for E2 and Erns specific antibodies using CSFV specific marker ELISAs revealed no CSFV specific antibodies in vaccinated pigs at PID 0 with 4 out the 5 kits used, indicating no cross-reaction to the antibodies induced by the chimeric virus. However, two of the CSFV specific ELISA kits, including the one that detected E2 at PID 0, showed a cross reaction when testing a BVDV positive sample. This means that these two kits can not be used as companion tests for the CP7_E2gif based vaccine. Using the three other CSFV specific kits E2- and Erns antibodies could be detected at 7 days and/or 14 days, as well as 42 days post challenge infection, respectively, depending on the specific kit used.

In conclusion, this study shows that several CSFV specific ELISAs can be used as companion tests together with the CP7_E2gif vaccine for screening purposes. Detailed results will be presented on a poster at the EPIZONE third annual meeting 2009.

The data originated from a joint EPIZONE CSFV DIVA-ringtrial and results from this inter-laboratory test study will be presented at the EPIZONE third annual meeting by Schroeder et al.

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Rasmussen, T.B, Reimann, I., Beer, M. and Uttenthal, A. (2008): DIVA properties of the chimeric pestivirus CP7_E2gif. Poster presented at the EPIZONE second annual meeting in Brescia.

# POSTER: LACK OF NETWORK INFORMATION MAY BIAS REGIONAL PREDICTIONS OF INFECTIOUS DISEASE TRANSMISSION

LYYTIKAINEN, TAPANI[1]; SAHLSTROM, LEENA[1]; VIRTANEN, TERHI[1]; KALLIO, EVA[2]

FINNISH FOOD SAFETY AUTHORITY EVIRA[1]; UNIVERSITY OF LIVERPOOL[2]

Key words: Network Information, Predictions, Infectious diseases, Regionality, Pig

The magnitude of an outbreak of infectious disease, such as foot-and-mouth disease (FMD), is partly dependent on the number and the type of contacts the first (primary) infected farm has during the infective period. Also other farms have influence on the final size of epidemic outbreak and thus they should be included in when the transmission of a disease is predicted.

The Finnish FMD Monte Carlo simulation model was applied to estimate the spread of FMD among the Finnish pig farm population (n=3228). Farm locations and pig transport network in Finland were applied to the FMD transmission simulations so that the target farms were selected depending on the primary farm. Data for this structured network simulations was based on animal movement registry and farm registry on 2006. The simulated epidemics (n=900 000) were compared to a model, which was otherwise similar, but where so-called random network simulation was used, i.e. the information on the contact farms was ignored. The magnitude of an epidemic outbreak was analysed in relation to the pig farm density, to evaluate the spatial implications of network information.

The outcomes of the two models deviated clearly when the results of primary farms were compared. The average epidemic size based on the random network simulation was different from the value of the structured network simulation for 40 % of the farms. Overestimations of the average epidemic size were more common than underestimations, when random network was used in the simulation. Underestimations by random network were approximately two times more common in the high density areas of pig production than in other parts of the country, but the overestimations were evenly distributed across the country.

The results of the study highlight the importance of the structured network information when the size of an epidemic is predicted. Thus, using detailed network information in simulations will improve accuracy of predictions. Underestimations by the random network were concentrated in regions with the highest pig farm density in Finland. This indicates that in these regions the networks may amplify the spread of disease above the level, which could be predicted solely by the contact structure and contact frequency of the primary infected farm. To conclude, network structure should be taken into consideration when predicted epidemic sizes are used in contingency planning in these areas.

# POSTER: MOLECULAR CHARACTERIZATION OF VIRULENT NEWCASTLE DISEASE VIRUSES ISOLATED IN MALI IN 2007 AND 2008

GIL, PATRICIA[1]; SERVAN DE ALMEIDA, RENATA[1]; HAMMOUMI, SALIHA[1]; MOLIA, SOPHIE[1]; CHEVALIER, VERONIQUE[1]; TRAORE, ABDALLAH[2]; SAMAKE, KASSOUM[2]; ALBINA, EMMANUEL[1]

CENTRE DE COOPÉRATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT (CIRAD)[1]; LABORATOIRE CENTRAL VÉTÉRINAIRE MALI[2]

Key words: Newcastle disease virus, Mali, phylogenetic analysis

Newcastle disease (ND) is still one of the most important diseases in the poultry production worldwide in spite of vaccination measures applied since more than fifty years. Different isolates of Newcastle disease virus (NDV) have been grouped in avirulent, intermediately virulent and virulent viruses depending on the type of clinical signs and severity of disease. Based on the analysis of nucleotide sequence of the F protein gene, 10 different genotypes (I-X) (1) or 6 different lineages (1-6) (2) of NDV have been identified to date. The genotypes VI (lineage 4) and VII (lineage 5) were further divided into seven (VI or 4a-g) and five (VII or 5a-e) subgenotypes/sublinages, respectively (1). In Africa ND causes important economical damages in poultry and is endemic in several countries. However, the genotype distribution of NDV strains as well as the protection level conferred by vaccination are still poorly understood in this continent.

In this study, isolates of NDV from healthy chickens were collected from various locations in Mali during a surveillance programme in 2007 and 2008. Nucleotide sequencing and phylogenetic analyses of positive samples were conducted on 1659 nucleotides of the F gene, including the cleavage site of the F protein, and on the full sequence of the HN gene. For this purpose, cloacal and tracheal swabs were collected from healthy poultry in different areas of Mali during 2007 and 2008. After the detection of the NDV by real time PCR, all NDV positive samples were inoculated into the allantoic cavities of 9-11 days-old embryonated SPF fowls' eggs. Four isolates were obtained after two passages. After extraction of viral RNA, complementary cDNA was synthesized and 5 distinct conventional PCR were used to generate the complete sequence of the F and HN genes. The sequencing of the F gene showed that the 4 Malian isolates contain a virulent cleavage site (G/RRRKR/FV or G/RRQKR/FI) with at least 3 basic amino-acids (Arginine, R). The presence of V118 associated with the cleavage site G/RRRKR/FV in two of these isolates is rare and was only recently reported in the neighbouring country Burkina Faso (3). According to the sequence analyses, the strains isolated in one site (Mopti market for example), in the same bird species at the same period are identical but differ from the strains isolated in another site (Sikasso) in the next year. Moreover, the phylogenetic analysis of these 4 isolates shows that they are closed to the genotype VII (or lineage 5) which represents the currently circulating genotype in Europe, Asia and Africa (4), but with some motifs of other genotypes like V118, characteristic of genotype V (lineage 3). Snoeck et al. (3) suggested that NDV isolates from West Africa, genetically distant from all known sublineages, represent three new ones (tentatively named by the authors 5f, 5g and 5h). It is possible that the Malian NDV isolates are clustering into the new sub-genotypes circulating in Nigeria and Burkina Faso supporting that these sublineages represent the NDV variants indigenous to West Africa.

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# POSTER: INVESTIGATION OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS (CCHF) IN TICKS IN TURKEY

ALBAYRAK, HARUN[1]; ÖZAN, EMRE[1]; KURT, MITAT[1]

SAMSUN VETERINARY CONTROL AND RESEARCH INSTITUTE[1]

Key words: Antigen, CCHF, ELISA, Tick, Turkey

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus of the genus Nairovirus in the family Bunyaviridae. This virus is endemic in sub-Saharan Africa, the Middle East, and southern Eurasia. Transmission to humans is primarily through the bite of an infected Ixodid tick, most commonly of the Hyalomma genus, or direct contact with blood or tissues from infected humans or livestock.

In this study, a total of 1790 adult ticks were collected from cattle (302 pools, 782 ticks in total), sheep (81 pools, 636 ticks in total), goat (36 pools, 357 ticks in total), buffalo (1 pool, 1 tick in total) and turtle (1 pool, 14 ticks in total) in coastal and inland of Middle and East Blacksea Region (Samsun, Sinop, Ordu, Giresun, Tokat, Amasya, Sivas) in Turkey. According to tick size, the pools range from 1 to 20 ticks. 13 different tick species (Hyalomma marginatum marginatum ticks (106 pools, 1060 ticks in total), Hyalomma anatolicum excavatum ticks (32 pools, 320 ticks in total), Hyalomma anatolicum anatolicum ticks (2 pools, 20 ticks in total), Hyalomma detritum ticks (28 pools, 280 ticks in total), Hyalomma aegyptium ticks (1 pools, 12 ticks in total), Rhipicephalus bursa ticks (86 pools, 860 ticks in total), Rhipicephalus turanicus ticks (103 pools, 1030 ticks in total), Ixodes ricinus ticks (58 pools, 580 ticks in total), Haemaphysalis punctata ticks (1 pools, 10 ticks in total), Haemaphysalis sulcata ticks (1 pools, 10 ticks in total), Dermacentor marginatus ticks (3 pools, 30 ticks in total)) were recognized on the animals in the region..

Tick pools were tested by ELISA for the presence of Crimean-Congo hemorrhagic fever virus (CCHFV). Virus antigen was found in %10.92 (46/421) of the pools. According to this result; positivity rate of provinces were detected 33.87% (21/62), 4.34% (1/23), 8,86% (7/79), 6,09% (5/82), 7.40% (4/54), 5.08% (3/59), 8.06% (5/62), 8.06% for Samsun, Ordu, Giresun, Sinop, Amasya, Tokat and Sivas respectively.

CCHFV antigen was detected from 7 tick species. Except buffalo and turtle, viral antigen was detected from ticks found on cattle, sheep and goat.

# POSTER: AFRICAN SWINE FEVER VIRUS IN CAUCASUS

KOLBASOV, DENIS[1]; TSYBANOV, SODNOM[1]; KURINNOV, VIKTOR[1]; KALABEKOV, ISMAIL[1]; ELSUKOVA, ALEKSANDRA[1]; SHENDRIK, ALENA[1]

NATIONAL RESEARCH INSTITUTE OF VETERINARY VIROLOGY[1]

Key words: ASFV, PCR

African swine fever virus (ASFV) is a highly contagious and usually mortal porcine viral infection. There is no vaccine against ASF. The only control measure is slaughter of affected and suspicion swine population, urgent and correct diagnostic of this disease being an effective way to prevent the infection further spreading in both the Russian Federation and other countries, as far as the disease can be catastrophic for animal industry.

In spring 2007 ASF outbreaks Georgia were observed in 10 regions. A total of 20,000 pigs were culled. The outbreaks of African swine fever in Georgia have been registered in 10 areas of dense swine population, including Tbilisi. In these areas, some 20,000 infected pigs have been destroyed. Georgia has imposed restrictions upon movement of any livestock, contaminated premises have been disinfected, and a quarantine regime has been introduced in the disease-affected areas.

After that, ASFV has been detected in Azerbaijan on January 22, 2008. The virus was found there in a village Nidzh of Gabala area, which has been quarantined.

In June 2008 an ASF outbreak in North Ossetia (Russia) over 5,000 pigs were far killed. About 1,300 pigs died of the virus and more than 3,800 pigs have been culled in the republic since the outbreak was registered on June 30 in four of the province's eight districts.

An outbreak of African swine fever virus in South Russia's Stavropol Region was reported on October 15 in a village Gorkaya Balka at a farm containing over 6,000 pigs. Following the regional service report, the disease has so far killed as many as 115 pigs. Later the tests confirmed the deaths were caused by the ASF. Around 600 pigs will be culled in southern Russia to prevent the spread of African swine fever virus, which was detected in Stavropol Region.

Nowadays, the ASF diagnostics has been drastically improved. SRI NRIVVaMR has a wide range or effective methods to be used for the infection diagnostics, including PCR and real-time PCR tests. The PCR techniques have been developed using primers from a highly conserved region p72 of the genome, to detect and identify a wide range of isolates belonging to all the known virus genotypes. Both of these PCR techniques were used to investigate virus isolates taken from ASF disease foci in Russia, but real-time PCR method was more useful for ASFV detection because of its higher rapidity / sensitivity and improved diagnostic specificity. The PCR test systems we have developed in SRI NRIVVaMR are well adapted to RotorGene (Corbett Research), iQ5 (BioRad) and StepOnePlus (Applied Biosystems) real-time units which are preferably used in diagnostic laboratories in Russia.

# POSTER: DETECTION, ISOLATION AND IDENTIFICATION OF MYCOPLASMA CAPRICOLUM SUBSP. CAPRIPNEUMONIAE IN GOATS IN CHINA

CHU, YUEFENG[1]; GUO, HAN[1]; LU, ZHONGXIN[1]; ZHAO, PING[1]; GAO, PENGCHEN[1]; HE, YING[1]

LANZHOU VETERINARY RESEARCH INSTITUE (LVRI)[1]

Key words: Detection, Mycoplasma capricolum subsp. Capripneumoniae, epidemiology, China

M. capricolum subsp. caripneumoniae (Mccp) is generally considered to be the etiologic agent of contagious caprine pleuropneumonia (CCPP), an OIE list disease which leads to significant economic losses in many developing countries in Africa and Asia. CCPP has been described since the 1920s and reported in many provinces in China, but detection of the causative agent of the disease, Mccp, have rarely been reported. So there is very little detailed information on this disease in China.In this paper, pleural fluid and lung materials from two young goats in a herd of 216 local dairy goats suffered from severe respiratory disease and high flock mortality were used for direct PCR detection by the methods as described by Bölske et al (1996) and woubit et al (2004), and isolation of Mycoplasma species by inoculating in the modified Thiaucourt's medium. The fragments specific for Mccp were detected directly from pleural fluid and lung tissues and two strains of Mycoplasam designated M2301 and M1601 were isolated successfully from pleural fluid of both goats. The two isolates were clone purified for three times and subjected to biochemistry test, PCR and sequences comparison analysis. The results showed both of them belong to the Mccp species. Thus, the evidence that CCPP caused by Mccp is present in China was confirmed. On the other hand, the fact that the fragments specific for Mccp were detected directly from pleural fluid and lung tissues also indicated that the two PCR methods above can be the technologies for rapid diagnosis of Mccp, considering the difficulties for isolation of this mycoplsma species.

# POSTER: A MULTIPLEX-PCR FOR DETECTION OF HAEMOPHILUS PARASUIS, ACTINOBACCILLUS PLEUROPNEUMONIAE, PASTEURELLA MULTOCIDA

CHU, YUEFENG[1]; HE, YING[1]; LU, ZHONGXIN[1]; ZHAO, PING[1]; GAO, PENGCHEN[1]

LANZHOU VETERINARY RESEARCH INSTITUE (LVRI)[1]

Key words: multiplex-PCR, Haemophilus parasuis, Actinobaccillus pleuropneumoniae, Pasteurella multocida, diagnosis

A multiplex polymerase chain reaction (m-PCR) assay was optimized for the simultaneous detection of several species of important bacterial pathogens in the upper respiratory tract of swine. Three sets of oligonucleotide primers specific for Haemophilus parasuis(Hps), Actinobaccillus pleuropneumoniae(App), Pasteurella multocida(Pm) based on published single PCR methods were used in this test. The m-PCR was able to simultaneously amplify a 1090 bp fragment of Hps chromosomal DNA, a 342 bp fragment of App chromosomal DNA and a 457 bp fragment of Pm chromosomal DNA in one reaction. The test was evaluated on a collection of 50 field isolates (31 strains of Hps, 12 strains of App and 7 strains of Pm) as well as on reference strains of this bacteria (15 serotype reference strains of Hps and App, C44-8 strain of Pm). The m-PCR confirmed the identification of the reference strains as well as field isolates, and can detect minimum 100 cells of each species or 50 Pg of Hps and App DNA or 500pg of Pm DNA in one reaction. The sensitivity of the m-PCR is quite comparable with the reported uniplex PCRs. The test was further evaluated on 18 clinical samples (pleura, pericardium, lung tissues, joint and heart blood) from 5 pigs suffered from respiratory distress. 5 of 18 samples from 2 pigs were positive for the three bacteria, 6 of 18 samples were positive for both Hps and Pm, and 2, 3 of 18 were only positive for Pm and Hps, respectiverly. The results suggested the m-PCR assay is a potential tool for differentiation of the three pathogens and diagnosis of mixed infection by these bacteria.

# POSTER: THE SITUATION OF PPR IN MARMARA REGION OF TURKEY

UNSAL BACA, AYSEL [1]; GURBUZ, S.[1]; SAIT, A.[1]

PENDIK VETERINARY CONTROL AND RESEARCH INSTITUTE[1]

Key words: Peste des petits ruminants, RT-PCR, cELISA

Peste des petits ruminants (PPR), is a highly contagious transboundary viral disease of goats and sheep. PPR is an endemic disease in Turkey since the first outbreak, reported officially in 1999.
This report shows the situation of PPR in Marmara region between 2005-2008. Pendik Veterinary Control and Research Institute (PVCRI) is responsible to provide services to 12 provinces of Marmara region. Between the years of 2005-2008, a total of 413 PPR suspected case were monitored by both cELISA and RT-PCR for the presence of PPR virus in PVCRI. As a result; PPR virus was detected in 137 cases, out of 413 suspected episode accepted from both Thrace region and the rest of the Marmara region. It is a pleasant situation that a decrease was observed on the number of PPR outbreaks in recent years. It was thought that vaccination with homolog strain and control measures had a positive effect on the PPR outbreaks.

# POSTER: STABILITY OF MICRORNA IN PARTLY DEGRADED RNA EXTRACTED FROM LUNG TISSUE

SKOVGAARD, KERSTIN[1]; MORTENSEN, SHILA[1]; WENDT, KARIN T[1]; LAURITSEN, KLARA T.[1]; HEEGAARD, PETER M. H.[1]

TECHNICAL UNIVERSITY OF DENMARK [1]

Key words: RT-PCR, host response, microRNA, diagnostics

MicroRNAs have gained considerable interst as small non-coding RNA playing a prominent role in the post-transcriptional epigenetic regulation of gene expression. In the diagnostic field it is being investigated if expression profiles of these small non-coding RNAs can be used as a host signature or fingerprint to yield information on the nature of ongoing infections. In principle this involves the investigation of tissue to reveal correlations between the known infection status of an animal and the presence and composition of microRNAs in its tissue.

We investigated the usability of microRNAs for quantitative RT-PCR in partly degraded RNA isolated from lung tissue. The stability of microRNA was previously found to be relatively high in formalin-fixed paraffin-embedded tissue (Li et al., 2007). The small size of microRNA could account for the greater tolerance to degradation as compared to normal-sized mRNA (Fleige et al., 2006).

Lung tissue from three healthy pigs was cut into 1cm x 1cm pieces and stored at room temperature in Petri dishes for 0 h, 1 h, 8 h, 24 h, 48 h, and 72 h respectively. At the stated time points RNA Later (Invitrogen) was added to stabilise the RNA and total RNA (including small RNA) was extracted using TRI Regent (Sigma) and the integrity of the RNA was determined using two different chips (RNA Nano and Small RNA) on the Agilent Bioanalyser. A correlation coefficient of 0.87 was found between RNA degradation (RNA integrity number (RIN)) and time (h), confirming a linear increase with time in the degradation of RNA in the lung tissue at room temperature. During the time span of this study RIN decreased from 9.0 (±0.12) at time 0 h to 4.7 (±1.57) at time 72 h. Electropherograms of small RNA confirmed these results, ranging from high quality RNA samples, with a clear tRNA peak at time 0 h to highly degraded samples after 72 h.

The relative concentration of three well described reference genes (B2M, β-actin and GAPDH) was compared with the relative concentration of three putative microRNA reference genes: mir-23a, mir-26a and mir-34a at the six time points, using quantitative RT-PCR after total RNA extraction and quantification (Nanodrop spectrophotometry). SYBR green chemistry was used for mRNA as wellas microp RNA real time PCR quantification. The concentration of mir-23a, mir-26a and mir-34a was found to be relatively stable within the first 24 h., whereas the concentration of the three mRNA reference genes started to decrease within less than 8 h.

Previous studies have established that RNA stability is dependent on variation in the type and quantity of active ribonucleases as well as differences in tissue structure (Schoor et al., 2003; Seear and Sweeney, 2007). In this study we tested the stability of microRNA and mRNA in partly degraded RNA isolated from lung tissue. Initial results indicate that mir-23a, mir-26a and mir-34a are comparatively stable at room temperature in lung tissue within the first 24 h. after retrieval.

# POSTER: RAPID AND WIDELY DISSEMINATED ACUTE PHASE PROTEIN RESPONSE AFTER EXPERIMENTAL BACTERIAL INFECTION OF PIGS

SKOVGAARD, KERSTIN[1]; MORTENSEN, SHILA [1]; BOYE, METTE [1]; POULSEN, KARIN T[1]; CAMPBELL, FIONA M[2]; ECKERSALL, P DAVID[2]; HEEGAARD, PETER M H [1]

TECHNICAL UNIVERSITY OF DENMARK[1]; UNIVERSITY OF GLASGOW[2]

Key words: Pig acute phase response, Actinobacillus pleuropneumoniae

The acute phase protein response is a well-described generalized early host response to tissue injury, inflammation and infection, however its biological function(s) and its interplay with other innate host responses are not well-known. The response comprises pronounced changes in the concentrations of a number of circulating serum proteins, the so-called acute phase proteins, and in order to gain new insight into this response in the context of a bacterial infection we studied gene expression changes in peripheral lymphoid tissues as compared to hepatic expression changes 14-18 h after an experimental respiratory infection of pigs. The lung infection was established with the pig specific respiratory pathogen Actinobacillus pleuropneumoniae. Quantitative real-time PCR based expression analysis were performed on samples from liver, tracheobronchial lymph node, tonsils, spleen and on blood leukocytes, supplemented with measurements of interleukin-6 (IL-6) and selected acute phase proteins in serum.

C-reactive protein and serum amyloid A were clearly induced 14-18 h after infection. Extrahepatic expression of acute phase proteins was found to be dramatically altered as a result of the lung infection with an extrahepatic acute phase protein response occurring concomitantly with the hepatic response. This suggests that the acute phase protein response is a more disseminated systemic response than previously thought, involving tissues distant to the infection locus including immunoprivileged tissues such as tonsils. The current study provides to our knowledge the first example of porcine extrahepatic expression and regulation of C-reactive protein, haptoglobin, fibrinogen, PigMAP, and transferrin in peripheral lymphoid tissues.

# POSTER: RE-ACTIVE AND PRO-ACTIVE SURVEILLANCE FOR BAT LYSSAVIRUSES IN THE NETHERLANDS, AN UPDATE.

KOOI, BART[1]; LINA, PETER[2]; REUSKEN, CHANTAL[3]; VAN DER POEL, WIM[1]; KRAMPS, HANS[1]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]; NATURALIS MUSEUM OF NATURAL HISTORY[2]; NATIONAL INSTITUTE FOR PUBLIC HEALTH AND THE ENVIRONMENT[3]

Key words: bat lyssavirus surveillance EBLV

Bats are the most abundant and widely distributed non-human mammalian species in the world. Several bat species are reservoir hosts of zoonotic pathogens and therefore can be a potential threat to public health.
To assess future threats posed by zoonotic pathogens carried by bats, there is a need for accurate knowledge of the factors underlying disease emergence, and also for an effective surveillance programme and a rapid response system. Therefore effective re-active, as well as pro-active surveillance systems for EBLVs should be established.
Since 1984 such a reactive surveillance program is running in the Netherlands to investigate the presence of European Bat Lyssaviruses (EBLV-1 and EBLV-2) in bats. In this program all contact cases, with humans or pets, are send to the laboratory in Lelystad and tested for EBLV with an EU prescribed immune fluorescence test (IFT). To date more then 4000 samples have been tested. About 50% of the bats send in are Pipistrellus pipistrellus, the main bat species in the Netherlands, in which we did not find EBLV so far. The main reservoir for EBLV is Eptesicus serotinus, 298 positive cases so far (20%), second species is Myotis dasycneme, with almost 4% positives this far.
Since 2006 we have extended our survey with a proactive surveillance. In cooperation with national bat workers live bats are captured and sampled for saliva and for faeces, and if possible blood. So far the main species captured are Myotis dasycneme (n=156) and Myotis daubentonii (n=46), none of the samples were positive for EBLV-1. Detections of coronaviruses and EBLV-2 are ongoing.
Because of the increasing interest in bats as carriers of zoonotic pathogens we are now establishing a tissue bank in cooperation with other research institutes in the Netherlands. Current focus is on coronaviruses and Salmonella/Campylobacter.

# POSTER: IMMUNE-STIMULATORY PROPERTIES OF DENDRIMERS MULTIVALENTLY PRESENTING MURAMYLDIPEPTIDE

SORENSEN, NANNA S [1]; BOAS, ULRIK[1]; HEEGAARD, PETER[1]

TECHNICAL UNIVERSITY OF DENMARK[1]

Key words: dendrimer, adjuvant, PAMP, Muramyl dipeptide

Pathogen-associated molecular patterns (PAMPs) are evolutionarily conserved microbial molecules recognized by pattern-recognition receptors of dendritic cells, of which the Toll-like receptors are a prominent group. In general, PAMP-structures are composed of small repeating units. Binding of PAMPs to TLRs induces cells to express costimulatory molecules and inflammatory cytokines, enabling an inductive antigen-presentation. We wanted to elucidate if small PAMP-derived structures ("PAMP minimal essential motifs") could be used to produce molecularly well-defined adjuvants and vaccine delivery systems for the targeted delivery of antigens in an optimally immune-activating manner for the induction of tailored adaptive immune responses.

As a starting point it was investigated whether PAMPs might be mimicked by the multivalent presentation of PAMP minimal essential motifs on the surface of globular synthetic polymers, dendrimers. Polypropylene-imine (PPI) and polyamido-amine (PAMAM) dendrimers of two sizes (generations, G) were conjugated with the minimal essential motif of peptidoglycan (PGN), muramyldipeptide (MDP) to yield molecules presenting a theoretical number of 16 ($G_3$-MDP) and 32 ($G_4$-MDP) MDP molecules, verifying the conjugates by HPLC. These conjugates were tested for their in vitro cytotoxicity and for their ability to induce the production of cytokines and to up-regulate MHC and co-stimulatory molecules in porcine peripheral blood mononuclear cells, as compared to the effect of free MDP and unconjugated dendrimers at equivalent molar concentrations.

Both $G_3$- and $G_4$-MDP conjugates of PPI and PAMAM induced the production of IL-12 p40 at the highest concentration tested, and similar effects were seen with regard to production of IL-1β and IL-6. Unconjugated dendrimers did not induce cytokines to any significant degree, but some induction of cytokine production was seen with free MDP. Overall, the MDP-conjugated dendrimers of PAMAM type were particularly efficient inducers of all three cytokines and the $G_4$-MDP of PAMAM type induced IL-12 p40 and IL-6 production to the same level as did PGN. By the measurement of DNA-intercalating dye uptake using flow cytometry, enhanced cell death was detected in response to unconjugated $G_4$ PPI, but neither the unconjugated $G_3$ PPI or $G_4$ PAMAM nor any of the MDP-conjugated dendrimers showed any detectable cytotoxicity in this assay.

Using surface expression of $SWC_3$ and $SWC_1$ to gate the monocytes, we found that this population showed a marked up-regulation of both B7 and MHC class II by 24 hours of culture in medium only, indicating an activation of the cells by culture. However, after culture with PGN, the monocytes had down-regulated both markers. Interestingly, the MDP-conjugated dendrimers had an effect comparable to that of PGN on both activation markers, with the potency of down-regulation seen with the different conjugates corresponding to the cytokine responses seen with the same constructs.

# POSTER: PORCINE CIRCOVIRUS TYPE 2 (PCV2) IN CASES OF DIARRHOEA AND ENTERITIS IN PIGS

SZCZOTKA, A[1]; PODGORSKA, K[1]; ZMUDZKI, J[1]; KOZACZYNSKI, W[1]; PEJSAK, Z[1]; STADEJEK, T[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: PCV2, wild boar, diarrhoea

Introduction

The aim of the study was to analyze the presence of PCV2 in cases of antibiotic non-responsive diarrhoea and to evaluate the possible role of the virus in development of enteritis in pigs.

Material and methods

Internal organs and feces from 60 pigs, 5 – 19 weeks old, from 37 farrow-to-finish farms, PMWS-positive or PMWS-suspected. Sections of lymph nodes and intestines (ileum, caecum and colon) were analyzed for presence of PCV2 DNA by in situ hybridization test (ISH) (2). They were also hematoxilin-eosin (HE) stained for standard histopathological examination. Addtionally, fecal samples were tested for presence of B. hyodysenteriae and L. intracellularis by PCR (3).

Results

In samples from 35 pigs, 10 – 17 weeks old, large amounts of PCV2 DNA, typical for PMWS, were detected in lymph nodes by ISH. In this group, in samples from 17 pigs, PCV2 was also found in abundant amount in samples of ileum. The remaining 18 pigs, PCV2-positive in lymph nodes, were negative in ileum. In samples from only 1 animal lymph nodes were negative for PCV2 in ISH, but virus was detected in considerble amount in ileum. In HE stained sections of lymph nodes histopathological lesions characteristic for PMWS were identified. Similar lesions were observed in PCV2-positive samples of ileum. 31 samples of feces were negative in PCR for B. hyodysenteriae and L. intracellularis. DNA of L. intracellularis was found in feces from 1 pig and mixed infection was detected in 3 animals.

Discussion

According to the obtained results in PMWS-affected pigs similar lesions could be observed both in lymph nodes and in ileum and they correlate with clinical outcome of disease. Also, it was found that presence of PCV2 in ileum could be correlated with diarrhoea in PMWS- free animal. Detection of B. hyodysenteriae and L. intracellularis together with the absence of PCV2 show that although the emergence of PCV2 as an intestinal pathogen may represent a new phenomenon (1), in differential diagnosis other causative agents of diarrhoea must be take into consideration. In the animals negative for B. hyodysenteriae and L. intracellularis and PCV2 other causative agents of diarrhoea should considered. Because the study was performed on small number of samples, futher investigations need to be performed to confirm the role of PCV2 as an etiological agent of diarrhoea in pigs.

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# POSTER: PATHOGENICITY OF AVIAN INFLUENZA VIRUSES ISOLATED FROM MARMARA REGION

UNSAL BACA, AYSEL[1]; AKCADAG, B.[1]; IYISAN, A.S.[1]; TURAN, N.[1]; SAIT, A.[1]; FUCULAR, F.[1]; UN, H.[2]

PENDIK VETERINARY CONTROL AND RESEARCH INSTITUTE[1]; CENTRAL VETERINARY CONTROL AND RESEARCH INSTITUTE[2]

Key words: Pathogenicity, AI, H5N1, Marmara region

Avian Influenza (AI) or "Bird Flu" is a highly contagious viral infection of birds and can manifest itself in different ways, depending mainly on the pathogenicity of the virus and the species affected. The first outbreak of AI in Turkey was detected in a backyard poultry house on the 5th of October 2005 in province of Balıkesir.
This report represents the number of AI outbreaks and the pathogenicity of the isolates in Marmara Region. Between 2005-2008 fifteen AI case were detected as AI H5N1 out of 1446 case by virus isolation, HA,HI test, RT-PCR, and realtime RT-PCR. Further information concerning the pathogenicity or potential pathogenicity of the isolates were determined by sequencing the genome at the cleavage site of the haemagglutinin. The amino acid motif at the HAo cleavage site of fifteen isolates were the same as -PQGERRRKKRGLF- representative of high pathogenicity based on the repeated basic aminoacids. One AI sample isolated from a duck from the provinces of Samsun were detected as H7N1, revealed the amino acid motif at the HAO cleavage site representative of low pathogenicity. N1 sequences of a highly pathogen H5N1 and low pathogen H7N1 showed high levels of heterogenicity.

# POSTER: IMPROVE TOOLS AND STRATEGIES FOR THE PREVENTION AND CONTROL OF CLASSICAL SWINE FEVER

KOENEN, FRANK[1]; HAEGEMAN, ANDY[1]; UTTENTHAL, ÅSE[2]; LE POTIER, MARIE-FRÉDÉRIQUE[3]; KULCSÁR, GÁBOR[4]; LOEFFEN, WILLIE[5]; DURAN, JUAN PLANA[6]; BEER, MARTIN[7]; STAUBACH, CHRISTOPH [7]; DE MIA, GIAN MARIO[8]; ROSSI, SOPHIE[9]; GAO, RONG[10]; BELÁK, SÁNDOR[11]; MOENNIG, VOLKER[12]; SÁNCHEZ-VIZCAÍNO, JOSE MANUEL[13]; THULKE, HANS-HERMANN[14]; DELATER, NIELS[15]; CALISTRI, PAOLO[16]; HOFMANN, MARTIN[17]

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE (VAR)[1]; NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[2]; AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[3]; CENTRAL AGRICULTURAL OFFICE, DVMP[4]; CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[5]; FORT DODGE VETERINARIA S.A[6]; FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[7]; ISTITUTO ZOOPROFILATTICO SPERIMENTALE UMBRIA E MARCHE[8]; OFFICE NATIONAL DE LA CHASSE ET LA FAUNE SAUVAGE[9]; SICHUAN UNIVERSITY[10]; NATIONAL VETERINARY INSTITUTE (SVA)[11]; UNIVERSITY OF VETERINARY MEDICINE HANNOVER (HVS)[12]; UNIVERSIDAD COMPLUTENSE DE MADRID [13]; HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH[14]; SPECTOS GMBH[15]; ISTITUTO ZOOPROFILATTICO SPERIMENTALE ABRUZZO & MOLISE[16]; EIDGENÖSSICHES VOLKSWIRTSCHAFTSDEPARTEMENT[17]

Key words: Classical Swine Fever Virus, Live Marker Vaccine, Back yard pigs, DIVA diagnostics, Epidemiology

This abstract summarizes the aims and objectives of a newly submitted CSFV_goDIVA pro-ject
Although classical swine fever (CSF) has been eradicated in wide areas within the EU, the disease is still endemic in some wild boar populations and in domestic pigs in some of the new member states, particularly in back yard pigs. This increases the risk of CSF re-emergence and persistence in pig farms.

In order to improve the eradication strategies the project aims are a) the final devel-opment and testing of a live marker vaccine candidate for the prevention and improved con-trol of CSF, applicable both orally and intramuscularly; b) the development and optimisation of accompanying discriminatory diagnostic tests; c) the production of an effective oral deliv-ery system for the marker vaccine for use in wild boar and back yard pigs; d) the easy selec-tion of diseased animals using infra red temperature measurement; e) the evaluation of strat-egy concepts to control outbreaks in the wild or domestics while accounting for the new tools. The improved knowledge on immunological reactions and pathogenesis will support a more efficient vaccine application and provide data for the epidemiological models.
In addition, research about bait-uptake by wild boar will allow increasing the efficacy of oral vaccination.

Epidemiological studies of CSF in domestic pigs, back yard pigs, and in wild boar, in-cluding molecular epidemiology will increase the insight of CSF transmission and persis-tence. Epidemiological models will be developed to support risk assessment and early warn-ing systems, both for conventional eradication strategies as well as for new strategies using the new vaccines and diagnostic tools taking into account the role of CSF reservoirs.
A proof of concept concerning the suppression of viral replication by alternative methods will also be provided.

In conclusion, the new EU-funded project will provide the scientific basis for new strategies of eradication and control of CSFV in the European Union.

Acknowledgments:
This research project CSFV_goDIVA was submitted as "Small Collaborative Project" to the European Union, Brussels, in the framework of FP7 (Grant Nr.: 227003 CP-FP)
The support of the EU Network of Excellence, EPIZONE (Contract No FOOD-CT-2006-016236) was very helpful by the realization of the project submission.

# POSTER: CAN BE DIFFICULT THE DIAGNOSIS OF PESTE DES PETITS RUMINANTS (PPR) IN FUTURE: CHARACTERISTIC AND NONCHARACTERISTIC CLINICOPATHOLOGICAL FINDINGS IN THE SHEEP AND GOAT IN TURKEY

TOPLU, NIHAT[1]

ADNAN MENDERES UNIVERSITY[1]

Key words: peste des petits ruminants; PPR; sheep; goat; viral infection

The present study exhibit the observation of clinicopathological findings and immunohistochemical diagnosis of PPR in Aegean region, Turkey since 1999. As characteristic clinical findings, PPR usually show severe erosive, necrotic stomatitis and enterocolitis and pulmonary lesions. Although the oral cavity and intestinal lesions in clinical and postmortem examinations were absent or mild especially in adult animals (<6 months old), pulmonary findings were predominant in sheep and goat flocks. On the other hand, presense of syncytial cells and inclusion bodies, characteristic findings of PPR, was unequal in lesions of the oral cavity, intestines and lungs in histological examinations. Immunohistochemistry displayed viral antigen to be found especially in the tissues of oral cavities, intestinum, lungs and lymph nodes in both characteristic and noncharacteristic cases.
In conclusion, the present study exhibit that PPR was a variably presenting disease in respect to clinicopathological findings , and the diagnosis could be very difficult. Thus, practitioners and even pathologists should be aware of PPR when facing unidentified clinicopathological changes. Based on our laboratory results, we strongly suggest that immunohistochemistry should be used for certain diagnosis in tissues taken from PPR suspected animals.

# POSTER: IMMUNOPHENOTYPE OF BOVINE DENDRITIC CELLS GENERATED FROM MONOCYTES OF HEALTHY AND BLV INFECTED ANIMALS.

SZCZOTKA, M.[1]; KUZMAK, J.[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: dendritic cells, immunophenotype, bovine leukemia

Introduction

Dendritic cells (DCs) are professional antigen presenting cells (APC), which initiate primary immune responses and play an importat role in the generation of peripheral tolerance. Following their encounter with antigen or danger signals, DCs migrate to the lymph nodes, where they activate effector cells essential for tumour clearance. Although the DCs system is highly heterogenous, the differentiation and function of DCs populations is largely regulated by exogenous factors. Malignancies and retroviruses appear to exploit this by producing immunosuppressive factors capable to affect DCs, thus exerting systemic effects on immune response. The aim of this study was to isolate and characterize dendritic cells from healthy cattle and after infection with BLV.

Material and methods

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by density-gradient centrifugation (Histopaque, Sigma) and then monocytes were generated. CD14+ cells were isolated by positive selection, using super-paramagnetic particles labelled with antibodies to human CD14 (Miltenyi-Biotech). Then, cells were cultured in complete RPMI-1640 medium containing FCS, glutamax and gentamycine. To generate DCs, GM-CSF or rbGM-CSF and IL-4 were added to the cultures and cultivation was performed in incubator with $CO_2$ flow. After 10 days cells were harvested. Phenotypic characterization of DCs was performed by flow cytometry using different fluorochrome-conjugated antibodies: CD14, CD11a, CD11b, CD11c, MHC-I, MHC-II and CD13. Morphology of cells was determined after stainig with Giemsa stain.

Results

After immunomagnetic separation of CD14 cells it was found that purity of selection was about 92%. In the cell cultures different morphological types of DCs were observed and they were on different levels of maturity. It was detected that dendritic cells generated from normal blood cells had different CD markers in comparison to cells infected with BLV. In BLV infected animals very high expression of CD11b, CD11c, MHC-I and MHC-II was observed.

Discussion

There is no reliable method established for the isolation of bovine peripheral blood DCs and the phenotypes and the functions of bovine DCs are still not completely clear. The attempt of the present study was to identify bovine peripheral blood DCs by special selection.Immature myeloid DCs transmit this information to peripheral lymphoid tissues, where they relay it to T-lymphocytes. The results obtained during this experiment may be usefull in the knowledge of the mechanism of BLV infection.

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# POSTER: EPIDEMIOLOGY AND CONTROL OF CLASSICAL SWINE FEVER IN WILD BOAR AND POTENTIAL USE OF A NEWLY DEVELOPED LIVE MARKER VACCINE

KOENEN, FRANK[1]; HAEGEMAN, ANDY[1]; BELÁK, SÁNDOR[2]; BELÁK, KATINKA[2]; BEER, MARTIN[3]; KRAMER, MATTHIAS[3]; MOENNIG, VOLKER[4]; GUBERTI, VITTORIO[5]; HOFMANN, MARTIN[6]; DURAN, JUAN PLANA[7]; SÁNCHEZ-VIZCAÍNO, JOSE MANUEL[8]; KULCSÁR, GÁBOR[9]

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE (VAR)[1]; NATIONAL VETERINARY INSTITUTE (SVA)[2]; FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[3]; UNIVERSITY OF VETERINARY MEDICINE HANNOVER (HVS)[4]; ISTITUTO NAZIONALE PER LA FAUNA SELVATICA[5]; INSTITUT FÜR VIRUSKRANKHEITEN UND IMMUNPROPHYLAXE[6]; FORT DODGE VETERINARIA S.A[7]; UNIVERSIDAD COMPLUTENSE DE MADRID [8]; CENTRAL AGRICULTURAL OFFICE, DVMP[9]

Key words: Classical Swine Fever Virus, Live Marker Vaccine, Wild Boar, DIVA diagnostics, Epidemiology

The present presentation summarizes the results of a FP6 funded project.

Aim 1: Development of an epidemiological for CSF eradication in wild boar
A number of epidemiological parameters where either determined by using existing published methods, data collection or by new applications for estimating them (such as transmission coefficient β and the minimum wild boar number). For the latter a user-friendly excell sheet was developed based upon the hunting bag. Subsequently, a new mathematical model based upon a metapopulation principle was designed and validated using data from previous out-breaks. This new model showed that hunting is an ineffective way to control the infection as only unrealistically intensive hunting efforts could eradicate the infection. Although in small populations (<1000 to 1500 animals) a non-intervention policy revealed to be successful, vac-cination was demonstrated to be an effective tool in controlling the CSFV infection as it al-ways reduces the epidemic peak. The chance of successful eradication of the infection is de-termined by the percentage of the susceptible population that is vaccinated within a short range of time. While a 60% vaccination rate of susceptible animals will lead to prompt eradi-cation, 20% will increase the probability of endemic stability of the infection.

Aim 2: Adaptation of the C-strain vaccine baits for use in wild boar with special attention to young animals
New small spherical and cuboid baits were designed and constructed. The new 3 cm spherical bait clearly showed an improved uptake rate in young animals up to 3.5 months. However, even this new small bait was not taken up by animals younger then 3 months, probably due to the fact that they prefer suckling. This has important implications in any vaccination strategy as it has to be kept in mind that these young animals cannot be immunized in this way. Fur-thermore, it was demonstrated that lyophilization increased vaccine stability under field con-ditions and is therefore a promising method to increase bioavailability during vaccination campaigns.

Aim 3: Development of a marker vaccine and accompanying diagnostic assays and protocols
A new live marker vaccine was developed whereby the E2-region of BVDV (strain CP7) was replaced by the corresponding sequence of CSFV (Alfort 187). Based upon in vitro and in vivo results, it can be clearly stated that CP7_E2alf is the most suited vaccine candidate as it not only provides complete sterile immunity, independent of the application method, it is also very safe to use. In none of the animal experiments any adverse effects were noted on health or farrowing with normal birth performance and no effect on the health status of the piglets. Not withstanding the BVDV background of CP7_E2alf, no serological or virological evidence could be found for the presence of CP7_E2alf in young ruminants and rabbits upon oral appli-cation, and even intramuscular application of cattle and sheep did not result in detectable vac-cine virus replication or shedding. In addition to the safety of this candidate, animals immu-nised with CP7_E2alf can be differentiated from wild type infected animals using either commercial ELISA system or by a real-time RT-PCR developed during this project. The ro-bustness of the developed real-time RT-PCR was confirmed during an interlaboratory evalua-tion.

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# POSTER: POLISH WILD BOAR (SUS SCROFA) IS A RESEVOIR OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) BUT NOT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV)

SZCZOTKA, A[1]; PODGORSKA, K[1]; FABISIAK, M[2]; PEJSAK, Z[1]; STADEJEK, T[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]; WARSAW AGRICULTURAL UNIVERSITY[2]

Key words: wild boar, PCV2, PRRS, meat juice

Introduction
Postweaning multisystemic wasting syndrome (PMWS), porcine reproductive and respiratory syndrome (PRRS) and classical swine fever (CSF) are the most important virus diseases of swine. It was suggested that wild boars (Sus scrofa) might constitute a reservoir for etiological agents of these diseases, porcine circovirus type 2 (PCV2) and PRRS virus (PRRSV). The objectives of the study were to investigate presence of antibodies against PRRSV and PCV2 as well as genetic material of PCV2 in samples from wild boars.

Material and methods
The study was performed on diaphragm muscles and tonsils from wild boars hunted in 2007 and in 2008 in Poland. 209 tonsils samples and 145 samples of meat juice obtained in 2007 and 2008 were used. Samples of muscles were frozen at -20°C and after thawing meat juice was collected. The detection of specific IgG antibodies against PRRSV was performed using indirect in house ELISA test. The detection of specific IgG and IgM antibodies against PCV2 was performed on meat juice samples using Ingezim Circovirus IgG/IgM ELISA test (Ingenasa, Madrid, Spain). Additionally, these samples were analyzed for the presence of E2 glycoprotein of classical swine fever virus (CSFV) in Chekit-CSF-sero (Bommelli Diagnostics, Bonn, Switzerland) ELISA. DNA extracted from tonsils was real-time PCR amplified for the presence of PCV2 capsid protein gene (cap) (1094-1566 bp) (2).

Results
PCV2 specific IgG and IgM antibodies were detected in 69 out of 145 (47.6%) wild boars. An antibody profile suggesting a recent infection (IgM value < IgG value) was detected in only 4 animals (2.8%). Remaining 63 (43.4%) wild boars had high levels of IgG and negative IgM values suggesting old infection. Active infection was present in 2 (1.4%) animals. All the samples were negative in PRRSV ELISA. There was no indication of antibodies against CSF. PCV2 DNA was present in 83 samples.

Discussion
The results indicate high seroprevalence to PCV2 in Polish wild boar, which may suggest an endemic status. These findings are in agreement with studies by Vincente et al. (5). No antibodies against PRRSV were detected, which indicates that, like in Spain (3) and Slovenia (4), PRRSV has little or no importance in Polish wild boars population. Also, our small-scale results concerning CSFV in meat juice are consistent with those obtained in NVRI in wild boars sera monitoring (Lipowski – personal communication). Although it is known that wild boar can be important vectors in spread of variety of swine infectious diseases (1), this study did not show the significant role of wild boars in spreading of PRRS and their role in PCV2 infection remains to be investigated.

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# POSTER: GENETIC DIVERSITY OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IMPACTS THE SENSITIVITY OF PCR DIAGNOSTIC METHODS

STADEJEK, TOMASZ[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: PRRSV, PCR, diagnosis

Porcine reproductive and respiratory syndrome virus (PRRSV) is a ss RNA virus from the Arteriviridae family. There are two genotypes of this virus recognized: European (genotype 1) and North American (genotype 2). While genotype 2 constitutes of relatively genetically similar strains, genotype 1 is highly divergent and constitutes of multiple, relatively distantly related genetic subtypes. Interestingly only one of the subtypes (subtype 1) is known to be globally distributed while the remaining subtypes were found only in the East European countries formerly being part of the Soviet Union (Lithuania, Latvia, Belarus, Ukraine and Russian Federation) (2). The aim of the study was to compare the sensitivity of the selected PCR methods for the detection of different genetic variants of PRRSV.

Three PRRSV strains were used in the study, namely Lelystad (genotype 1, subtype 1), Aus (genotype 1, subtype 2) and Hesse (genotype 2). Additionally material from pig infected with PRRSV genotype 1, subtype 3 (from Belarus) and from pig infected with highly pathogenic variant of PRRSV genotype 2 (from Bhutan) were used. RNA was extracted using MagnaPure LC station and cDNA was synthesized with random nonamers. Next, 5 ul of tenfold dilutions of the cDNA was subjected to four different PCRs used in our laboratory (two for ORF5 and two for ORF7). One of the ORF5 specific assays was genotype discriminatory multiplex nested PCR (OLSU+UMOL), while the second one was nested PCR for universal detection of both genotypes (PEOL). One of the ORF7 specific assays was single step PCR (OL) (1) while the second employed OL amplification with the additional nestd PCR (OLST). For the nested PCR steps 1 ul of the first PCR product was used. Prior to PCR, primer annealing temperatures for each method were optimized using Hesse and Lelystad virus strains' cDNA. The same PCR reagents were used for all assays.

All PCR methods allowed detection of all used variants of PRRSV but significant differences in their sensitivity were observed. Generally, the sensitivity obtained in ORF7 targeted assays was from 10 to 10 000 times higher than the sensitivity of the ORF5 specific PCR assays for a given PRRSV variant. Nested PCR for ORF7 (OLST) had equal to 100 times higher sensitivity than single stage PCR for ORF7 (OL). Higher differences in the assays' sensitivity were observed for the detection of the genotype 1 than genotype 2 variants due to much higher diversity of the former genotype (2). The interpretation of the gel based PCR is performed based of the size of the product. The ORF5 size is highly conserved between and within the genotypes (603-606 nt). On the other hand ORF7 is highly polymorphic and can consist of 371to 393 nt. Such differences in size of ORF7 amplicons make difficult gel electrophoresis based assessment of the specificity of PCR. These preliminary results clearly indicate necessity of careful validation of the PCR methods for PRRSV diagnosis as well as interpretation of their results, especially for genotype 1 detection.

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# POSTER: MOLECULAR ANALYSIS OF THE E2 CODING REGION OF CSFV STRAINS

MALEK, B[1]; KAMIENIECKA, K[1]; PODGORSKA, K[1]; STADEJEK, T[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: CSFV, E2 protein, SK6

Introduction

Classical swine fever virus (CSFV) belongs to the Pestivirus genus of the Flaviviridae family together with bovine viral diarrhea virus (BVDV) and border disease virus (BDV) of sheep (1). Considering that the disease and its control have extremely serious economic consequences, CSFV has to be rapidly identified and traced. The ability to discriminate between CSFV strains is prerequisite for studies of the spread of the virus in the field and can be difficult for closely related isolates (2). Molecular epidemiology is important tool for tracing virus spread (4).
The objective of present study was to compare phylogenetic analysis of two fragments of E2 protein coding region of CSFV vaccine strains and Polish CSFV isolates.

Materials and methods

The 11 isolates from Poland from 1992-1994 and 6 vaccine strains were used. The 1000 nt fragment of the genome coding for E2 protein was PCR amplified and sequenced (5). Phylogenetic analysis of this sequence as well as its most diverse 190 nt fragment was performed (3). The obtained sequences were compared to the representative set available in GenBank.

Results

Phylogenetic analysis revealed that all Polish field isolates belonged to group 2.3 CSFV. Analysed strain were highly similar to each other in the analysed fragment of the E2 protein coding region. The tested vaccine strains belonged to several genetic lineages and were located in groups 1.1 and 1.2.

Discussion and Conclusions

It was shown previously that 190 nt fragment (2500-2690) of E2 protein is very useful for phylogenetic analysis (3). However, analysis of such a short fragment not always allows for discrimination of closely related strains or isolates. In this work we expected that extension of the analysed fragment to 1000 nt will increase the resolution of the phylogenetic analysis. However, our results showed that for discrimination of closely related field isolates from small geographical area or even vaccine strains sequencing of other genomic regions may be required additionally to E2.

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# POSTER: EXPERIMENTAL INFECTIONS OF PIGS WITH H7 AND H5 AVIAN INFLUENZA VIRUSES

MORENO, ANA[1]; SOZZI, ENRICA[1]; LELLI, DAVIDE[1]; VINCO, LEONARDO JAMES[1]; LOMBARDI, GUERINO[1]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL' EMILIA ROMAGNA (IZSLER)[1]

Key words: H5 and H7 avian influenza viruses, experimental infection, pigs

Introduction
Pigs can play an important role in the genetic reassortment of influenza viruses (IVs) having receptors both for avian and human IVs. The ability of pigs to be infected with different IVs and act as intermediate hosts could depend of virus subtype. This study provides preliminary information on the susceptibility and potential capability for pigs to act as intermediate host for avian influenza viruses (AIV) of high and low pathogenicity (LPAI, HPAI).
Material and Methods
Viruses. A/Tk/It/2962/03, A/Ck/It/13474/99 isolated in the LPAI H7N3 epidemic in 2002-2004 and in the HPAI H7N1 epidemic in 1999-2000. A/Tk/It/90302/05 H5N2 isolated in the LPAI epidemic in 2005 and A/Swan/It/2684/06 HPAI H5N1 AIVs. They were propagated in SPF chicken embryonated eggs (CEE). Infectious dose(ID) was expressed in 50% egg infective dose (EID50).
Experimental infection. Four experiments (exp) were conducted using 60 day old SPF swine housed in BSL-3 laboratory facilities and infected intratracheally. Several days(D) post infection (PI) other pigs were introduced for contact infection. The experiment designs are:
Exp1: H7N3 LPAI ; EID50:107,5; 2 infected pigs (IP): Nasal swabs+ (NS) 2-6 DPI; 3 contact pigs (CP) introduced 3DPI: NS^ 4-6 DPI
Exp2: H7N1 HPAI; EID50:107,7; 2 IP: NS 2-5 DPI; 2 CP introduced 2DPI: NS 4-5 DPI
Exp3: H5N2 LPAI; EID50:108; 2 IP: NS 2-6 DPI; 1 CP introduced 0DPI: NS 2-5 DPI
Exp4: H5N1 HPAI; EID50:107,5; 6 IP: NS 2-6 DPI and tissue samples*: 2IP/2DPI and 2IP/4DPI; 2 CP introduced 0DPI: NS 4-6 DPI
+ days starting 2 DPI (once a day)
^ days starting 1 D after introduction (once a day)
* trachea, lungs, lymph nodes, heart, spleen, liver, kidney, small and large intestine and skeletal muscle
Sera: IP and CP 10, 17, 25, 32 DPI
Nasal swabs and tissue samples were tested in influenza type A real time RT-PCR(rRT-PCR)(2). Positive samples were further inoculated into CEE. Antibody titers were determined by nucleoprotein A competitive ELISA (NPA-EL) and virus neutralization(VN)tests(1,3).

Results
No animals showed respiratory signs. An increase of body temperature was reported 1 day PI in 2 pigs only,1 in exp2 and 1 in exp4. All nasal swabs from exp 1 and 3 were negative. In exp 2, nasal swabs of 1/2 infected pigs were positive by rRT-PCR on day 2, 3 and 4 PI however the virus was isolated only from that collected 4 days PI. Samples of other infected and contact pigs resulted negative. In exp4, all nasal swabs were negative by rRT-PCR. The RNA was evidenced only in lungs and lymph nodes of 1 pig sacrificed 2 days PI but virus was recovered only from lungs. All pigs challenged with LP and HP H7 and LP H5 AIVs had antibodies against NPA and homologous HA. Serological response was observed: 10 and 17 days PI in exp 1, 10 days PI in exp 2 and 10 and 25 days in exp 3. In exp 4, only 1 infected animal resulted positive to NPA-EL and VN from 10 days PI.

Discussion
The results prove that pigs can be infected by both LP and HP AIVs but can not transmit infection to contact animals. Despite the high ID used, pigs showed low susceptibility to infection with the 4 AIVs. Virus shedding and detection were demonstrated only in exp with HPAVs. However positive serological response evidenced on all infected pigs in exp1, 2, 3 confirmed virus replication. In exp four 4/6 infected pigs remained negative. Further investigations will be needed for a better understanding of the ecology of AIVs.

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# POSTER: PRELIMINARY VALIDATION OF THE ID SCREEN INFLUENZA H7 COMPETITIVE ANTIBODY ELISA

POURQUIER, PHILIPPE[1]; LESCEU, STEPHANIE[1]

IDVET[1]

Key words: Influenza H7 ELISA immunodiagnostics

1. Introduction and Objectives

Given the recent outbreaks of Influenza H7 avian influenza in Europe, ID VET has developed a competitive ELISA for the detection of anti-H7 antibodies in bird sera. This preliminary study evaluates the specificity, sensitivity, and analytical sensitivity of the test.

2.Material and Methods

The ID Screen® H7 ELISA was used according to the manufacturer's instructions for the analysis of:

- Chicken (N=31), turkey (N=28) and duck (N=38) field sera from healthy animals (non-vaccinated, non-infected)
-15 influenza-negative, NDV-positive sera
-8 non-H7 Influenza strains
-796 H5N1 and 478 H5N2 vaccinated chicken sera from Egypt; 50 H5N2/H5N1 vaccinated then naturally-infected sera from Egypt were also tested.
-4 positive H7 sera, kindly provided by Intervet and Merial, tested by both IHA and H7 ELISA.

Analytical sensitivity was tested through the analysis of dilutions of an H7 reference serum from IZS Padova and an H7N1 vaccinated serum from Merial, France.

3.Results

1. 109/112 Influenza-negative field sera tested were found negative, giving a specificity of 97,32% (IC95:91.79% – 99.3%).
2. 1384/1418 H5-vaccinated sera were found negative, giving a specificity of 97,6% (IC95:96.62% – 98.31%).
3. All non-H7 influenza strains tested were found negative.
4. All H7 sera tested were found positive. These sera were tested in dilution by ELISA and IHA. The kit was calibrated in order to detect dilutions giving IHA titres of 1/4 to 1/8. The analytical sensitivity of the kit should be slightly higher than IHA.
5. Titrations of the Intervet and Merial sera gave positive results for dilutions of at least 1/125 and 1/2560, respectively.

4. Discussion and Conclusion

The ID Screen® Influenza H7 Antibody Competition ELISA demonstrates excellent specificity when tested on negative field samples and H5-vaccinated samples. Serotype specificity is also high.
Supplementary sensitivity studies are required in order to complete kit validation. It is, however, difficult to access positive H7 sera due to the fact that infected animals are rapidly culled in the European Union. ID VET welcomes any collaborations with laboratories having such sera in their possession.

# POSTER: PRELIMINARY VALIDATION OF THE ID SCREEN INFLUENZA H5 COMPETITIVE ANTIBODY ELISA

POURQUIER, PHILIPPE[1]; LESCEU, STEPHANIE[1]

IDVET[1]

Key words: Influenza H5 ELISA immunodiagnostics

1. Introduction and Objectives
Given the recent outbreaks of Influenza H5 avian influenza in Europe, ID VET has developed a competitive ELISA for the detection of anti-H5 antibodies in bird sera. This preliminary study evaluates the specificity, sensitivity, and analytical sensitivity of the test.

2.Material and Methods
The ID Screen® H5 ELISA was used according to the manufacturer's instructions for the analysis of:
- Chicken (N=36) and turkey (N=28) field sera from healthy animals (non-vaccinated, non-infected)
- 15 influenza-negative, NDV-positive sera
- 12 non-H5 Influenza strains
- 3 sera from animals naturally-infected by the H5 Influenza virus
- H5-vaccinated sera, including:
o 793 H5N1 vaccinated Egyptian chicken sera; of which 470 were also tested by HAI.
o 473 H5N2 vaccinated Egyptian chicken sera; of which 100 were also tested by HAI.
o 96 H5N1 and H5N2 vaccinated Egyptian chicken sera; of which 16 were also tested by HAI.
o 20 H5N2/H5N1 vaccinated then naturally-infected Egyptain sera were also tested. All 20 sera were also tested by HAI.
Analytical sensitivity was tested through the analysis of dilutions of an H5 reference serum from IZS Padova.

3.Results
1. All 64 Influenza-negative field sera were found negative, giving a specificity of 100% (CI95: 94.22% - 100%).
2. All non-H5 influenza strains tested were found negative.
3. All 3 sera from naturally-infected H5 animals were found positive.
4. 1333 /1382 Egyptian vaccinated sera tested were found positive, (doubtful sera were considered negative). Sensitivity on vaccinated sera was measured to be
96.32% (IC95: 95.15% – 97.22%).
5. For the Egyptian sera which were also tested by IHA, the relative sensitivity of the ELISA test was determined to be 584/602 = 97.01% (IC95: 95.22% - 98.17%).

4. Discussion and Conclusion
- Specificity of the ID Screen ELISA was 100% on the negative samples tested.
- On vaccinated samples, sensitivity was measured to be 96.32%. The vaccinated animals not detected by ELISA may concern animals which were not really vaccinated, or to animals which did not seroconvert, or to mis-labelled samples.
- Correlation between ELISA and HAI (Figure 4) was high but not perfect (the relative sensitivity of the IDVET kit was calculated at 97.01%). It is important to note that discordances between techniques are frequently observed due, in part, to differences in the type of antibodies detected by each technique. In this study, the relative ELISA sensitivity was high, and it is rare to achieve 100% correlation in this type of study.
- Access to non-vaccinated, naturally-infected sera was limited, due to the fact that infected animals are rapidly culled in the European Union. ID VET welcomes any collaborations with laboratories having such sera in their possession. Additional validation work is currently underway in Germany further to the 2008 LPAI H5 outbreak. Preliminary results are encouraging.

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# POSTER: HIGHLY PATHOGENIC VARIANT OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN BHUTAN

STADEJEK, TOMASZ[1]; RAIKA, VIJAY[2]; CHABROS, KATARZYNA[1]; RINZIN, KARMA[2]; PARCHARIYANON, SUJIRA[3]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]; NATIONAL CENTRE FOR ANIMAL HEALTH[2]; NATIONAL INSTITUTE OF ANIMAL HEALTH[3]

Key words: PRRSV, diagnosis

Porcine reproductive and respiratory syndrome (PRRS) is a virus disease of pigs that emerged in the end of 1980-ties. The disease it endemic in most of pig producing countries but occasionally it is observed in epidemic form with high mortality. Recently highly acute PRRS outbreaks were reported from China and Vietnam (1). These outbreaks increased global interest about this disease, including Europe. The highly virulent strains presently circulating in China and Vietnam pose serious threat for the neighboring countries. Here we describe the first case of PRRS in Bhutan caused by the highly pathogenic variant of PRRSV.

The nucleus pig herd at the National Pig Breeding Centre (NPBC), Wangchutaba, Bhutan was established in 2003 from the stock imported from the UK. On August 4, 2008, clinical symptoms resembling PRRS were observed in several sows. In August, 15 of 41 pregnant sows (36.5%) aborted on 52-109 day of gestation and two of them died. Three boars and two gilts also died. From August through October 15 further sows (36.5%) born litters consisting of stillborn and weak piglets. Post-mortem examination of the dead pigs showed congestion in lungs with interstitial pneumonia and hemorrhages in all organs. Twenty eight of 49 piglets born life in this period died before weaning. Serum samples from pigs from the affected herd were found to contain PRRSV specific antibodies. To characterize PRRSV from Bhutan, sera from 30 pigs and organs from four fetuses and two dead pigs were submitted to the OIE Reference Laboratory for PRRS at the National Veterinary Research Institute, Pulawy, Poland.
Antibodies specific for PRRSV genotype 2 were found in sera from 14 pigs (2). By PCR PRRSV genotype 2 was found in two of these sera and in one sample from seronegative sow. In three of six organ samples PRRSVs RNA was also detected. Results of PCR to detect specific 90 nt deletion in nsp2 coding fragment, considered the marker of highly pathogenic PRRSV strains from China and Vietnam confirmed presence of such deletion in the sequences from Bhutan (1). Sequence analysis of the amplicons showed that they were highly identical (99.7-100% at ORF5; 99.2-100% at ORF7). Their identity to the highly pathogenic strains isolated earlier in China and Vietnam (1) was also very high (98.8-99.7% at ORF5; 98.7-99.7% at ORF7). In the phylogenetic trees the sequences from Bhutan clustered together with those from China and Vietnam what supports their common origin. The identity of the sequences from Bhutan to the prototype PRRSV genotype 2 strain VR2332 was much lower ranging 88.6-88.9% at ORF5 and 93.3-94.1% at ORF7. The presented data indicate that the first PRRS outbreak in Bhutan was caused by the recently emerged highly pathogenic PRRSV mutant.

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# POSTER: GENERATION OF DNA VACCINES CO-EXPRESSING FMDV EPITOPES AND ANTI-APOPTOTIC PROTEINS

GULCE IZ, SULTAN[1]; DELILOGLU GURHAN, S. ISMET [1]; BORREGO, BELEN [2]; DOSKAYA, MERT [3]; GURUZ, YUKSEL[3]; SOBRINO, F[4]; ESCIBANO, JM[4]; RODRIGUEZ, FERNANDO[5]

EGE UNIVERSITY, DEPARTMENT OF BIOENGINEERING, [1]; CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[2]; EGE UNIVERSITY, FACULTY OF MEDICINE[3]; CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA[4]; CENTRE DE RECERCA EN SANITAT ANIMAL[5]

Key words: FMDV, DNA vaccine, Anti-apoptotic proteins

Conventionally inactivated FMD vaccines have been shown to be protective. However, several inconveniences such as their short safety period, the difficulty to distinguish between infected and vaccinated animals and the risk of working with large amounts of live-virus during vaccine production, have to be taken in to consideration. Therefore, the development of a new generation of safe immunogenic and protective FMDV vaccines is desirable. Borrego et al., showed that DNA vaccines based on B and T epitopes of FMDV C isolate can protect mice even in the absence of specific antibodies before challenging [1]. The aim of the present study is to construct a DNA vaccine co-expressing FMDV antigens and anti-apoptotic proteins. FMDV antigens used in this study were B epitopes of FMDV C (VP1; 133-156 aa), FMDV O1K (VP1; 131-157 aa) strains, in addition to T epitopes (VP4; 20-34 aa, 3A; 11-40 aa) of the FMDV C isolate. Aiming to increase the efficacy of the vaccine FMDV epitopes were fused downstream to a single chain variable fragment of an antibody that recognizes swine MHC class II antigens (scFv). Anti-apoptotic proteins used in this study were Bcl-xL of the host animal and the 3CD gene region of FMDV. While the addition of either Bcl-xL or the FMDV-3CD gene should increase the half-life of the antigen presenting cells co-expressing the B and T FMDV epitopes [2,3] thus enhancing the specific immune responses induced, the induction of a specific response against 3CD might also contribute to protect the host against the FMDV challenge. Preliminary studies about the vaccine generation and their expression upon transient in vitro transfection will be evaluated during the presentation.

# POSTER: BIO-SEEQ PORTABLE VETERINARY DIAGNOSTICS SYSTEM: FIELD-BASED PCR TESTING WITH AUTOMATED SAMPLE PREPARATION.

CZAJKA, JOHN[1]; VOLPE, CARMELO[1]; GREEN, DOUG[1]; BETLEY, JASON[1]; LEWINGTON, JAY[1]

SMITHS DETECTION DIAGNOSTICS[1]

Key words: Diagnostics, Pen-Side, PCR, Sample Preparation, Multiplexed

Introduction

Smiths Detection Diagnostics has developed the Bio-Seeq Portable Veterinary Diagnostics System, a portable PCR instrument specifically for field-based veterinary and environmental monitoring. The Bio-Seeq System has three core components; the instrument, the Sample Preparation Unit/Reagent Pack, and Linear After The Exponential (LATE) PCR. The device has five independently controlled thermocyclers, each of which has four optical channels, allowing for highly multiplexed reactions, and the device is water-tight, which allows for decontamination via immersion. The Sample Preparation Unit (SPU) is a single-use consumable that is driven by the instrument and extracts nucleic acids from the sample. The assay-specific Reagent Pack contains lyophilized reagents that are stable for over one year at room temperature. The system also utilizes Linear After The Exponential (LATE) PCR, a novel PCR amplification methodology that has excellent sensitivity and enables the reliable multiplexing of 10 – 20 target organisms.

Materials & Methods

The BioSeeq System was designed to provide "sample in, answer out" PCR testing for field veterinarians. Knowledge of PCR and molecular biology are not required and the sample preparation unit has been designed to work on a wide range of samples. Operation of the BioSeeq System is simple. The user obtains the sample to be tested (blood, epithelial tissue, swab), inserts the Reagent Pack into the SPU, and loads the sample into the open port on the SPU. The SPU is then capped and the barcode on the Reagent Pack scanned on the instrument. The instrument will then direct the user to insert the SPU into one of the thermocyclers, after which, the user can then enter sample information, such as an identification number, and presses "start" on the instrument. The BioSeeq System will then proceed with sample lysis, nucleic acid extraction, PCR amplification, and automated endpoint analysis of the assay. Results are displayed as positive or negative on the system display. If the identify of the pathogen can be determined, such as sub type or strain, it will also be displayed.

Results

Smiths Detection has successfully developed a portable PCR system that provide true "sample in, answer out" capability. Each of the core components, the instrument, SPU, and LATE-PCR, have demonstrated equivalent or superior performance compared to existing technologies that are commonly used for laboratory-based PCR testing. The first assay to be launched on the Bio-Seeq Portable Veterinary Diagnostics System was the pan-Foot and Mouth Disease Virus (FMDV) assay. The pan-FMDV assay has been evaluated with several strains of each of the seven FMDV serotypes, other viruses that cause vesicular disease, and sample spiked with purified FMDV RNA. Performance of the pan-FMDV assay, on laboratory PCR instruments, and shows very good equivalence to the gold standard PCR assay currently in use at the Institute for Animal Health, Pirbright. Testing on the BioSeeq Portable Veterinary Diagnostics System is to begin shortly.

Discussions & Conclusions

The BioSeeq System will enable veterinarians to perform laboratory-quality PCR testing in the field. This capability will assist veterinarians in treating infected animals in a more efficient and timely manner, as well as providing the ability to rapidly respond in outbreak situations, resulting in better outbreak management and containment.

# POSTER: SERODIAGNOSIS OF PESTIVIRUSES INFECTION BY COMPETITIVE-ELISA BASED ON MONOCLONAL ANTIBODIES AND RECOMBINANT NS3

PEZZONI, GIULIA[1]; STERCOLI, LIDIA [1]; CORDIOLI, PAOLO [1]; BROCCHI, EMILIANA[1]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL' EMILIA ROMAGNA (IZSLER)[1]

Key words: Pestiviruses, Antibody detection, ELISA, recombinant NS3, Monoclonal Antibodies

Bovine Viral Diarrhea Virus (BVDV), Border Disease virus (BDV) and Classical Swine Fever virus (CSFV) belong to Pestivirus group; the three viruses are antigenically related.
The most immunogenic and conserved protein among pestiviruses is the non structural protein 3 (NS3), a multifunctional enzyme with at least two domains associated with enzymatic activities: a serine protease activity and an NTPase-elicase activity.
Currently, serological assays for Pestiviruses are based on the detection of antibodies against NS3.
We expressed the NS3-NTPase-elicase domain (rNS3E) of BVDV NADL strain in a baculovirus/insect cell system, in order to preserve the natural antigenic and structural properties of the native antigen. The rNS3E is produced at high yield and is recognized in immunofluorescence by three anti-NS3 monoclonal antibodies (MAbs) previously raised against an Italian isolate of BVDV. According to the results of reciprocal competition the MAbs identify three different epitopes on the rNS3E as well as on the native antigen; their absence of reactivity in Western-blot indicates that the three epitopes are conformation-dependent. When combined as catching and conjugated antibody in sandwich ELISA assays, the three MAbs showed reactivity profiles with the rNS3E identical to those observed with the native antigen.
Overall, this reactivity provided evidence that the recombinant protein reproduces folding and antigenicity of the native viral protein, providing the conditions for the development of a functional antibody-detection ELISA for pestiviruses.
For the test design we chose a competitive ELISA, since it is equivalently applicable to any animal species. In particular, the MAb-based competitive test, routinely used in our laboratory, was modified in order to substitute the BVD virus used as source of NS3 with the rNS3E. MAb 3H4 was confirmed as the best antigen capture antibody to display most antigenic epitopes and MAb 3A3, conjugated with peroxidase, as the best competing antibody.
Diagnostic performance of the competitive ELISA based on rNS3E was evaluated by testing bovine and pig sera in parallel with the in-house test for pestiviruses antibodies; an additional test for CSFV-specific antibodies, provided by the National Reference Laboratory for CSF (IZS Perugia), was used as confirmatory test for positive pig sera.
Results for 369 bovine sera showed a 100% concordance between the two ELISAs for pestiviruses, with 147 positive and 222 negative samples in both tests. Regarding pig sera, out of 2691 field samples examined, 221 scored positive and 2456 scored negative in both ELISAs (99.5 % concordant results). All the positive pig sera were elicited by infection with pestiviruses other than CSFV, as none of them reacted in the CSFV-specific ELISA.
The capability of recombinant competitive ELISA to recognize also infection by CSF virus was investigated using a panel of sera from pigs experimentally vaccinated with the CSF China strain and subsequently challenged with a pathogenic virus: results of the immune response detected by the recombinant ELISA consistently correlated with results provided by the in-house test based on the native viral antigen and by the CSFV-specific ELISA.
In conclusion, the rNS3E can successfully substitute the viral antigen in serological diagnosis of infections caused by pestiviruses; the use of a recombinant antigen in association with characterized MAbs makes the production and yield of biological reagents safer and easier and ensures improved standardization and reproducibility of serological tests.

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# POSTER: DETECTION AND SEROTYPING OF BLUETONGUE VIRUS BY MICROARRAY

ABU-MEDIAN, ABU-BAKR[1]; P. DUKES, JULIET[1]; MAAN, SUSHILA[1]; WATSON, MICHAEL[1]; P.C. MERTENS, PETER[1]; P. KING, DONALD[1]; BRITTON, PAUL[1]

INSTITUTE FOR ANIMAL HEALTH (IAH)[1]

Key words: Virus; Detection; Serotype; Microarray; Bluetongue

Bluetongue (BT) is now considered an important emerging transboundary disease in northern Europe. The situation is complex as multiple serotypes are now circulating in the region: in addition to the large number of outbreaks in 2006-08 due to BT virus (BTV) serotype 8, recent incursions of two further serotypes (BTV-1 and BTV-6) have occurred. In order to monitor and control the spread of BTV, it is essential to rapidly identify the serotype(s) causing a specific outbreak.

Existing parallel assay techniques to characterise BTV are time-consuming. A great advantage of a diagnostic microarray is that a single field or clinical sample can be analysed for the presence of multiple viruses in a single operation without prior knowledge of the identity of the pathogens. As part of a UK government-funded project (BioChip: http://www.bio-chip.co.uk) and EPIZONE (WP4.4), 3 generations of in-house oligonucleotide-based microarrays have been developed. The third generation microarray comprised 2884 oligonucleotide probes (70-mer) derived from 308 virus species from 36 families. Owing to the success of previous generations of this array with detecting and typing veterinary viruses such as the avian coronavirus, infectious bronchitis virus and the picornavirus, foot-and-mouth disease virus, and the threat imposed by BTV, 117 BTV-derived probes were designed using publicly available software and included in the array. The 'serotyping' probes were derived from an alignment of VP2 nucleotide sequences of all 24 BTV serotypes. From the alignments, a consensus or serotyped sequence was created. The probes were designed from two 200-base target regions. To create 'pan-tropic' BTV probes and probes capable of discriminating between eastern and western strains, segment 1 nucleotide data was used as a template.

In order to test the array, reference strains of the 24 BTV serotypes (provided by the OIE Reference Laboratory at the Onderstepoort Veterinary Institute, South Africa) were passaged in the BHK-21 cells, and total RNA was extracted and reverse-transcribed. The resulting cDNA was randomly amplified and labelled with Cy3-dCTP, and hybridised for four hours with the probes printed onto slides. Slides were scanned and fluorescence was quantified using scanner software. Raw data were normalised, statistically analysed and visualised using 'DetectiV' custom software (http://www.biochip-deteciv.co.uk). Analyses of raw data following scanning of microarrays hybridised independently with labelled cDNA from different BTV serotypes revealed different hybridisation profiles between the different serotypes with each serotype specifically detected. This study shows that a detection/serotyping microarray offers a rapid diagnostic and surveillance tool enabling the differentiation between circulating and vaccine serotypes of BTV.

# POSTER: DOMESTIC DOGS SITUATION FOR ECHINOCOCCUS MULTILOCULARIS IN A HIGHLY ENDEMIC DEPARTMENT OF FRANCE.

UMHANG, GERALD[1]; RATON, VINCENT[2]; HORMAZ, VANESSA[1]; SCHEREFFER, JEAN LUC[1]; BOUCHER, JEAN MARC[1]; CAILLOT, CHRISTOPHE[1]; COMBES, BENOIT[2]; BOUE, FRANCK[1]

AGENCE FRANÇAISE DE SECURITÉ SANITAIRE DES ALIMENTS, NANCY LERRPAS (AFSSA)[1]; ENTENTE INTERDEPARTEMENTALE DE LUTTE CONTRE LA RAGE ET AUTRES ZOONOSES[2]

Key words: Echinococcus multilocularis, dogs, helminths, tænid eggs, France

Alveolar echinococcosis is a parasitic zoonose caused by the fox tapeworm Echinococcus multilocularis. The parasitic cycle is predominantly sylvatic involving red foxes as definitive host and several species of small mammals as intermediate host. An expansion of endemic areas is actually observed across all Europe. The North-East French department of Meuse is highly endemic with a fox prevalence estimated at 41.3% in 2007. Most of the parasites biomass occurs in foxes, although domestic dogs, cats, and other species are also sporadically infected.
According to veterinarians we have organised a collect of dog faeces after praziquantel treatment. Four hundred and ninety three dogs faeces were collected by the dog owners during march-april 2008 divided onto the department. Intestinal helminths eggs have been isolated from the faeces using the flotation technique on $ZnCl_2$. Each samples has been analysed on microscope to determine the presence or absence of tænid eggs. On the positives samples, the identification of the tænid eggs species has been done by PCR amplification with JB11-12 primers that are specific of the NADH1 gene. The sequence analysis of the amplicon allows the determination of the species.
The different helminths observed are : Trichuris vulpis (7.1%), Ancylostomatidae (7.1%), Toxocara sp. (4.5%), Tænia sp. (0.8%), Toxascaris leonina (0.4%), Mesocestoïdes sp. (0.2%), Strongyloides sp. (0.2%), and Capillaria sp. (0.2%).
After analyse of Tænid eggs by PCR and sequencing we identified a T. crassiceps worm for the 4 dogs with tænids infections. One is a hunting dog, wormed twice a year with praziquantel molecule and the three others pet dogs, from the same owner, are not wormed. These dogs are predatory of rodents and they could be potentially infected by E. multilocularis.
There is no evidence of the fox tapeworm in this canine population but some risk dogs have been identified. According to our results the vermifugation of domestic dogs to prevent echinococcosis contaminations is not well adapted. In endemic region of France, hunting dogs and pet dogs should be considered as a potential vector for transmission of alveolar echinococcosis to humans.

# POSTER: FURTHER INVESTIGATIONS ON THE ORIGINS OF FOOT-AND-MOUTH DISEASE VIRUS OUTBREAKS IN EUROPE SINCE 1960

KNOWLES, NICK[1]; WADSWORTH, JEMMA[1]

INSTITUTE FOR ANIMAL HEALTH (IAH)[1]

Key words: Aphthovirus; foot-and-mouth disease; epidemiology; sequencing

Vaccination against foot-and-mouth disease (FMD) virus ceased in Europe in 1991 following the eradication of the disease. However, approximately 14 FMD outbreaks have occurred since that time and their origins have recently been described by Valarcher et al. (Transboundary and Emerging Diseases 2008; 55, 14-34) who used phylogenetic analyses of VP1 gene sequences. Prior to this, Beck and Strohmaier (Journal of Virology 1987; 61, 1621-1629), again using VP1 nucleotide sequencing, had attempted to trace the origins of FMD outbreaks in Europe between 1960 and 1985. We have extended these studies to include other previously unsequenced viruses from FMD outbreaks from 1960 onwards. As Beck and Strohmaier had previously found, many of the outbreaks appeared to be of vaccine or laboratory origin, however, from time-to-time viruses from outside Europe were detected. However, the lack of resolution of VP1 sequences (639 nt) meant that the precise origin of very closely related viruses could not be definitely determined. We suggest that complete genome sequences (~8200 nt) could possibly resolve these relationships, as has recently been demonstrated for FMD outbreaks in the United Kingdom in 2001 (Cottam et al.; Journal of Virology 2006; 80, 11274–11282) and 2007 (Cottam et al.; PLoS Pathogens 4(4): e1000050 doi:10.1371/journal.ppat.1000050).

# POSTER: RANGE EXTENSION AND CONTAMINATION GRADIENTS OF ECHINOCOCCUS MULTILOCULARIS IN FRANCE.

BOUE, FRANCK[1]; SCHEREFFER, JEAN LUC[1]; BOUCHER, JEAN MARC[1]; UMHANG, GÉRALD[1]; FAVIER, STEPHANIE[2]; RAOUL, FRANCIS[3]; GIRAUDOUX, PATRICK[3]; COMBES, BENOIT[2]; CLIQUET, FLORENCE[1]

AGENCE FRANÇAISE DE SECURITÉ SANITAIRE DES ALIMENTS, NANCY LERRPAS (AFSSA)[1]; ENTENTE INTERDEPARTEMENTALE DE LUTTE CONTRE LA RAGE ET AUTRES ZOONOSES[2]; UNIVERSITY OF FRANCHE-COMTÉ[3]

Key words: Echinococcus multilocularis, Fox, ELISA, France

Among the carnivore pathogens transmissible to human, the intestinal parasite members of Cestode class are very important. The Cestode from Echinococcus genus are transmitted to human by accidental ingestion of infective eggs, distributed with faeces of the definitive host, that are particularly resistant in the environment.
In most European endemic areas the cycle of Em is predominantly sylvatic involving red foxes as definitive host and several species of small mammals as intermediate host. Important factors enhancing the risk of exposure for humans include an increasing prevalence and number of infective eggs of Em shed in the environment by foxes.
In view of the probable public health significance of Em, the causative agent of human alveolar echinococcosis, there is an urgent need for identification of new endemic areas. Knowledge of area of repartition of the Em in France must be actualised to establish a pertinent program of fox surveillance and determine a baseline data to anticipate human risks of contamination in unknown endemic areas.
The aim of our study was to evaluate the emergence of the parasite, in a large area of Eastern of France, and to determine its geographical distribution. Base on grassland covering, red fox faeces have been sampled between 2000 and 2007 and parasitological examinations have been done using ELISA coproantigen detection test. During the period of 2000-2007, a total of 5854 fox faeces were collected from the field in 41 departments according to our sampling protocol. Each department have sent more than 90% of the expected number of samples. The majority of the faeces were collected during the two first winters 2000/2001 and 2001/2002, and correspond to 70 % of the total number of samples analysed.

A total of, 221 samples were considered as positives and 5149 as negatives. We found positive samples in 38 departments out the 41 departments investigated, which cover a large east area of France. For some of these departments it was the first time of detection.
The estimated apparent prevalence vary from 0.75% in Allier to 14.63% in the Territoire-de-Belfort and we where unable to detect E. multilocularis positives samples only in 3 departments.
According to these observation results we postulate that Em could be considered as emerging parasite in the fox population in the Western part of France but this expansion cannot really be proven due to the lack of adequate retrospective studies in newly recognized endemic arreas. This phenomenon could be related to the migration of infected red foxes that have the potential to spread infective Em eggs far from the area of detection to new places where the cycle could easily be establish. However, taking into account the level of infection in red foxes and the presence of pasture favourable for the common voles that are considered as major intermediate host in West of France, it is not surprising to find new positive areas.

# POSTER: CORRELATION OF BOVINE IMMUNODEFICIENCY VIRUS INFECTION AND BRUCELLOSIS IN DAIRY CATTLE IN ESFAHAN AND SHAHREKORD AREAS, IRAN

MAHZOUNIEH, MOHAMMADREZA[1]; MOKHTARI, AZAM[1]; KHAKSAR, KHADIJE [1]; FROSSARD, JEAN-PIERRE[2]

SHAHREKORD UNIVERSITY[1]; VETERINARY LABORATORIES AGENCY (VLA)[2]

Key words: BIV, Brucellosis, Iran

In spite of more than 30 years control program including test and cull and vaccination, the brucellosis is still endemic in Iran and many Mediterranean countries and is one of the most important zoonotic diseases. The eradication of intracellular bacteria is largely dependent on cell-mediated immunity, so we hypothesized that Bovine immunodeficiency virus (BIV) infection may play a role in the emergence of infectious diseases with immunosuppressant effects.

Dual infections with brucella and BIV in dairy cattle were tested using 367 brucella-seropositive, and 132 seronegative sera among 83 dairy farms in Esfahan and Shahrekord, Iran. Among 490 cattle, 4.5% of cattle were BIV-seropositive, so these results report the first BIV infection in Iran. The BIV-seropositive cattles were 3% (4 of 133), 4.5% (6 of 132) and 5.1% (12 of 235) among negative, low titre and high titre for anti-brucella antibodies respectively. Although there were no significant differences among these groups ($p > 0.5$), we found a high incidence of co-infection in Shahrekord cattle with high anti-brucella antibodies titre. The prevalence of BIV infections were 9.1% in Shahrekord brucella-seropositive cattles. In contrast, among 115 dairy cattle of Esfahan only 1.74% were BIV-seropositive. The co-infection in some farms suggests a correlation between brucella and BIV infections. Based on these results BIV may increases the susceptibility of animals to brucella infection and failure in vaccination. It needs to investigate more about the role of BIV, as a new emerging disease, in predisposing to other infectious agents like tuberculosis, John's diseases and economic loss.

# POSTER: BLUETONGUE VIRUS (BTV)-8: CLONING OF FULL-LENGTH GENOME SEGMENTS NS1, NS2, NS3, VP2, VP3, VP4, VP5, VP6 AND VP7

PETKOVIC, SONJA[1]; HOFFMANN, BERND[1]; KEIL, GÜNTHER[1]; VAHLENKAMP, THOMAS[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: BTV-8

1. Introduction and Objectives

Bluetongue (BT) is a viral disease of ruminants transmitted by Cullicoides species. Since 2006 Bluetongue virus (BTV)-8 spread in Europe to many countries including Germany, the Netherlands, Belgium, France, Denmark, Switzerland, the Czeck Republik and the UK. Until now more than 20.600 BTV-8 cases are reported only in Germany. Ten ds-RNA segments form the viral genome including seven structural (VP1-VP7) and three non-structural (NS1-NS3) proteins (Mertens, 1989; Roy, 1989, 2005). The objective of the project is to molecularly clone full-length segments of BTV-8 in order to generate recombinant viruses. The results of these investigations should lead to a better understanding of disease pathogenesis and to generate DIVA-vaccines with serotype cross-protecting properties.

2. Material and Methods

Using a reverse genetic system all non structural proteins and VP2 to VP7 were cloned in pX8ΔT. BTV-8 alignments from the Netherlands 2006 were used to design primers compatible to the 5' (GUUAAA) and 3' (ACUUAC) ends of all ten RNA segments containing recognition sites for EcoRI, PstI, HindIII, and BamHI. After sequencing the clones were cloned in pGEX -4T-1 including recognition sites for EcoRI and BamHI for further expression in eukaryotic cells.

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3. Results

We amplified, cloned in pX8ΔT and sequenced BTV-8 full length genome segments encoding all non-structural and all structural proteins except for VP1. In addition to that we amplified NS1- NS3 using specific primers for pGEX-4T-1 and cloned NS2 and NS3 by now in prokaryotic expression vectors.

4. Discussion and Conclusions

We cloned all segments encoding the non-structural proteins which are involved in viral replication, the formation of inclusion bodies, and viral release. Furthermore, we cloned VP2, VP3, VP4, VP5, VP6, and VP7 which show functions like viral capping and helicase activities, and are part of the viral capsid. We currently work on cloning several segments for comparative expression studies in eukaryotic cells.

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# POSTER: COMPARISON OF LIGHT TRAPS FOR COLLECTION OF ADULT CULICOIDES IN THE BALEARIC ISLANDS AND SOUTH AFRICA.

MIRANDA-CHUECA, MIGUEL ANGEL[1]; DEL RIO-LOPEZ, RICARDO[1]; MONERRIS-MASCARO, MIRIAM[1]; PAREDES-ESQUIVEL, CLAUDIA[1]; MIQUEL-AMER, MARGALIDA[1]; BORRAS-BORRAS, DAVID[2]; CALVETE, CARLOS[3]; VENTER, GERT[4]; ESTRADA, AGUSTIN[5]; LUCIENTES, JAVIER[5]

UNIVERSIDAD ISLAS BALEARES[1]; INSTITUTO DE BIOLOGIA ANIMAL, ISLAS BALEARES[2]; CENTRO INVESTIGACION Y TECNOLOGIA AGROALIMENTARIA[3]; ONDERSTEPOORT VETERINARY INSTITUTE[4]; UNIVERSIDAD DE ZARAGOZA[5]

Key words: Culicoides, Bluetongue, AHS,

Several species of Culicoides biting midges (Diptera; Ceratopogonidae) were shown to be involved in the transmission of a variety of pathogens, the economically most important being the orbiviruses that cause bluetongue (BT) and African horse sickness (Mellor et al., 1985). Susceptible species of Culicoides become infected after feeding on a viraemic host. After replication in the salivary glands, viral transmission can occur during subsequent feedings on susceptible vertebrate hosts.

Bluetongue affected Balearic Islands during 2000 and 2003, and since 2004 outbreaks were relative wide spread in the Iberian Peninsula (Calvete et al., 2006). Thus, BT is a constant threat for the agriculture sector. In mainland Europe, BT affects the countries of the Mediterranean basin and is expanding northwards as far as Belgium and The Netherlands (Gloster et al., 2007).

Around 20 species of Culicoides are considered as potential biological vectors of BTV (OIE, 1998; Mellor, 1990). Culicoides imicola are a proven vector of BTV and are considered the main species responsible for the transmission of the virus in Africa, the Mediterranean and southern Europe. However, the occurrence of BT in northern Europe, in the absence of C. imicola, indicate other Culicoides species e.g, C. obsoletus s.s., C. scoticus, C. dewulfi and C. pulicaris to also be involved in the transmission of this virus. It is also more than likely that other species of Culicoides will be added to this list in the near future.

Surveillance programmes, to determine the activity period and distribution of the vectors, has been developed and conducted in all European countries affected by BT. The main tools that were used were different models of down draught light traps.

The aim of this study was to compare the efficiency and specificity of the different traps used in different countries for the collection of Culicoides adults. The tested traps were; miniCDC (USA), Onderstepoort trap (South Africa), Rieb trap (France) and the UK trap. Light trap comparisons were carried out during autumn 2007 and during spring and autumn 2008 on a cattle farm in the Balearic Islands. These traps were also compared in South Africa during periods of high (summer) and low (winter) Culicoides abundance. Comparison between traps showed important differences in collecting Culicoides, and indicated the Onderstepoort trap, from South Africa, to be the most efficient trap in the autumn season. The CDC trap, however, was the most efficient trap in the spring season. The Onderstepoort trap also, seemed to be the most sensitive trap during periods of low vector abundance. In regards to species composition no significant differences were found.

The average number of Culicoides collected per night with the Onderstepoort trap was significantly higher during both periods. In periods with high Culicoides abundance the CDC trap collect significant more midges than the either the Rieb or Pirbright trap. In periods of low Culicoides abundance, there was now significant difference in the number of midges collected in the Rieb or CDC trap. Although all the traps indicate C. imicola to be the most abundant livestock associated Culicoides species in South Africa significant differences were found in the species composition, parous rates, sex ratios and Culicoides to other insect ratios as determined by the various traps. It will be important to evaluate these biases in trapping methods.

# POSTER: EPIDEMIOLOGICAL STUDY OF BLUETONGUE IN SOUTHERN SPAIN DURING 2007

GARCIA, I. [1]; ALLEPUZ, A.[1]; GONZÁLEZ, M. A.[2]; CASAL, J.[1]; NAPP, S.[1]; CARBONERO, A.[2]; BORGE, C.[2]; ARENAS, A.[2]

CENTRE DE RECERCA EN SANITAT ANIMAL[1]; UNIVERSIDAD DE CÓRDOBA[2]

Key words: Bluetongue, epidemiology, Spain

After the epidemic of BTV-4 in 2004, which affected Andalusia (Southern Spain), a new serotype (BTV-1) appeared in this region in 2007. During the following months, the virus spread quickly and 7,916 outbreaks were detected in Spain in 2007. Still, most of the outbreaks (56%) were detected in Andalusia (RASVE, 2008).

In this communication we present the results of the epidemiological and spatial study of the outbreaks that affected Andalusia during 2007.

Data on BT outbreaks in domestic animals in Andalusia were obtained from the Spanish Ministry of Agriculture, Fisheries and Food (RASVE, 2008). Information relative to epidemiological data (general characteristics of the farms, clinical sings, mortality and morbidity), were collected from the affected farms through personal interview with farmers.

A total of 4,436 outbreaks of BTV-1 were confirmed during that year: 3,162 in sheep flocks, 113 in goat flocks, 7 in cattle herds and 1,154 in mixed farms (sheep, goat and/or cattle in the same farm). The temporal distribution of the outbreaks presented a clear peak of infection between October and November.

The prevalence of positive farms to the RT-PCR analysis was 17.7%, and the within farm prevalence was 16.8%. The mortality rate in the affected farms was 8.6%. The most common clinical signs were: fever, depression, lethargy, facial edema, and salivation (observed in more than 70% of the infected farms). Lesions in oral mucosa, lameness and dyspnea were also observed.

The infected farms were located mainly in the western Andalusia, which coincided with the areas with both highest ruminant census and highest captures of C.imicola. Spatial models are being built in order to analyze the spatial distribution of the disease and more detailed results will be presented during the meeting.

# POSTER: A DIFFERENTIAL PCR APPROACH TO MONITOR CLASSICAL SWINE FEVER VIRUS CHALLENGE STRAINS DURING EXPERIMENTAL INFECTION OF C-STRAIN VACCINATED PIGS.

EVERETT, HELEN[1]; SOSAN, OLUBUKOLA[1]; CROOKE, HELEN[1]

VETERINARY LABORATORIES AGENCY (VLA)[1]

Key words: CSFV, C-strain, differential PCR, LNA probe

Classical Swine Fever Virus (CSFV), a Pestivirus in the family Flaviviridae, causes a severe hemorrhagic disease of pigs that has considerable economic and welfare consequences. The live, attenuated C-strain is a safe and effective vaccine, but its use in the EU is banned, except during emergency situations, owing to an inability to distinguish vaccinated and infected animals by serological testing. Detection of the vaccine virus in blood by real-time RT-PCR has been reported, but only transiently in some samples at early times post vaccination (1). This raises the possibility that real-time RT-PCR could be used to monitor a population of vaccinated animals for the presence of field virus to assist control of future outbreaks if, for example, live attenuated vaccine were used in a ring-fence, vaccinate-to-kill strategy to reduce virus dissemination. To learn more about how the C-strain vaccine would perform in such a strategy, we are studying the virological parameters associated with C-strain vaccination of pigs followed by intranasal challenge with the field strain, UK2000 7.1, which caused the most recent outbreak of CSFV in the UK. One of our aims is to assess ability to detect field virus in vaccinated animals using a real-time RT-PCR approach.

Ten-week old pigs, were vaccinated by intramuscular injection of 100 PD50 of the Reimser C strain vaccine according to the manufactures protocol. Viraemia was below the limit of detection of a conventional real-time RT-PCR (2) but low levels of vaccine virus could occasionally be detected in lymphoid tissue. In contrast, the UK2000 7.1 strain could readily be detected in blood and tissues of animals following intranasal inoculation. To characterize the amount of challenge verses vaccine virus in samples from animals that have been vaccinated and then exposed to the UK2000 strain, we have developed a differential real-time PCR that distinguishes between the vaccine and this challenge strain. This PCR is based on an amplicon within the Npro gene region and uses separate locked nucleic acid (LNA) probes to distinguish the two strains. The PCR is specific for the strains in question and is being applied to blood and clinical tissue extracts from animals that have been challenged at very early time points post vaccination in order to assess the usefulness of real time RT-PCR for monitoring virus during a vaccination campaign and to examine the distribution of virus following vaccination and challenge.

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# POSTER: RESULTS OF A VACCINATION / CHALLENGE STUDY WITH PORCILIS® CSF LIVE, A GPE- BASED VACCINE AGAINST CLASSICAL SWINE FEVER VIRUS (CSFV)

MEINDL-BOEHMER, ALEXANDRA [1]; BLOME, SANDRA[2]; DEIKE, STEFANIE[1]; MOENNIG, VOLKER[1]

UNIVERSITY OF VETERINARY MEDICINE HANNOVER (HVS)[1]; FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[2]

Key words: Classical Swine Fever, modified live vaccines, Porcilis® CSF Live, vaccination/ challenge trial

Classical swine fever (CSF) is one of the most important viral diseases of pigs and causes serious economic losses worldwide. Whereas a strict non-vaccination policy is pursued within the EC where vaccines may only be used in case of emergency, vaccination is a very effective strategy in order to minimize economic losses and/ or to eradicate the disease in countries where the disease is endemic.

Current vaccination programmes involve modified live vaccines (MLVs) and a subunit marker vaccine. The advantage of MLVs in comparison to the marker vaccine is that they induce a reliable immunity very fast and are considerably cheaper. In addition to the best known and characterized MLV based on C-strain several other vaccines based on distinct CSFV strains are available and are employed in different parts of the world (e.g. Thiverval, GPE-, PAV 250). Although these vaccines are used in practice, their characteristics have not yet been thoroughly described and for some of them very little data concerning their performance against contemporary CSF strains are available.

The aim of the study described here was therefore to gain more insight and to gather experimental data on the characteristics and performance of the vaccine based on the Japanese GPE- (guinea pig exaltation negative) strain of CSF (Porcilis CSF® Live, Intervet). For this purpose a vaccination/ challenge experiment was performed. Five weaner pigs were vaccinated with Porcilis CSF® (10^3 TCID50/pig) and three animals remained untreated and served as controls. Six weeks post vaccination of group 1 all eight pigs were infected intranasally and intramuscularly with 10^4 TCID50 of the moderately to highly virulent CSFV strain 634, Vi 3837/38 (genetic group 2.3* Uelzen). Animals of both groups were monitored daily for clinical symptoms according to the standard protocol used at HVS and body temperatures were taken. During the experiment native and EDTA blood samples were collected regularly. Four weeks post challenge all surviving animals were euthanized. Leukocyte counts, virus isolation, RT-PCR, antigen and antibody-ELISAs as well as various neutralization peroxidase-linked antibody assays were performed on each of the samples.

The results of this experimental trial showed that Porcilis® CSF Live was able to protect the vaccinated pigs from a lethal infection with CSFV isolate 634. Whereas all non-vaccinated control pigs died due to an infection with CSFV, none of the vaccinated pigs displayed any clinical signs of disease. At no time of the experiment – neither post vaccination nor post infection – it was possible to isolate viable CSF virus from the vaccinated animals. From the vaccinated group only two samples collected at 4 or 6 days post challenge were tested doubtful/negative in qRT-PCR. As virus isolation was negative, it is highly unlikely that these animals shed the virus. From 21 days post vaccination on, neutralising antibody titres against CSFV were found in all animals belonging to group 1. By the time of challenge infection these antibody titres had reached sufficient heights to protect the animals from an infection with CSFV.

The results of this study indicate that Porcilis® CSF Live leads to an effective immune response in vaccinated animals. To further elucidate the onset of immunity or to determine the role of humoral versus cellular-based immunity further studies will be needed.

# POSTER: CONCURRENT CONGENITAL PESTE DES PETITS RUMINANTS (PPR) AND PESTIVIRUS INFECTION IN NEWBORN LAMBS AND KIDS, AND ABORTED FETUSES

TOPLU, NIHAT [1]; OĞUZOĞLU, TUBA ÇIĞDEM[2]; ALBAYRAK, HARUN [3]

ADNAN MENDERES UNIVERSITY[1]; ANKARA UNIVERSITY[2]; SAMSUN VETERINARY CONTROL AND RESEARCH INSTITUTE[3]

Key words: peste des petits ruminants; PPR; pestivirus; sheep; goat

The present study describes the pathological findings, and immunohistochemical and reverse transcription-polymerase chain reaction (RT-PCR) diagnosis of PPR and concurrent pestivirus infection in 26 animals (24 lamb and 2 kid) from various flocks in Aegean and Black Sea regions, Turkey. Abortions of pregnant ewes and goats, births of lambs and kids with poor survival rate and nervous signs have been observed. At necropsy, erosive-ulcerative stomatitis, thymic hypoplasia, and occasional cerebellar hypoplasia and porencephaly were observed. Histopathologically, syncytial cells and inclusion bodies in the oral cavity mucosa and lungs proposed the presence of PPR in aborted and newborn animals, in support of immunohistochemistry and RT-PCR. Immunohistochemistry and RT-PCR also exhibited concurrent pestivirus infection in these animals. Brain tissues of the animals showed nonsuppurative meningoencephalomyelitis with hypomyelinogenesis. Immunohistochemically, pestivirus antigen was present in neurophil, neurons and glial cells in all portions of the brain. Moreover, PPR viral antigen was also detected in neurons, ependymal and meningeal cells in replicated brain sections.

In conclusion, PPR virus showed central nervous system involvement and neurotropism similar to other morbillivirus infections such as canine distemper and human measles. We think that PPR virus transmission to fetus may be via placentitis caused by the pestivirus infection. Moreover, it is also possible that neurotropism of PPR virus that meningoencephalitis were initially caused by the pestivirus that facilitates PPR virus transmission through injured blood-brain barrier to fetus.

# POSTER: A GOOD SYSTEM FOR EARLY DETECTION OF A DISEASE

FROEHLICH, ANDREAS[1]

FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[1]

Key words: surveillance, early diagnosis method

To understand not only the spreading but also the containment of an epidemic, it is essential to detect early a new or well-know disease in (specific) areas which have been free from this disease before. Various methods exist for this early detection. One of them is the target surveillance program which works with sentinel programs. Sets of sentinels are part of the population under risk and kept continuously under observation. In comparison with other strategies and under different conditions, the limits of sentinel programs will be demonstrated.

# POSTER: LARVAE-DERIVED PRODUCTION OF RECOMBINANT RIFT VALLEY FEVER VIRUS (RVFV) PROTEINS

BOSHRA, HANI[1]; GÓMEZ-SEBASTIÁN, SILVIA[2]; ESCRIBANO, JOSE-ANGEL M[2]; BRUN, ALEJANDRO[1]

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[1]; INSTITUTO NACIONAL DE INVESTIGACIÓN Y TECNOLOGÍA AGRARIA Y ALIMENTARIA[2]

Key words: Bunyavirus, Vaccine, Recombiant Proteins, Technique

Rift Valley Fever virus (RVFV), a member of the family Bunyaviridae, is an epizootic pathogen that has been identified in outbreaks ranging from sub-Saharan Africa to the Arabian peninsula. While infection of this virus is potentially fatal in humans, it primarily affects domesticated livestock, with a high mortality rate of young ruminants, along with abortion storms in affected herds.

While attenuated vaccines are currently available, their ability for widespread use is severely limited due to their high production costs, which are currently out of reach for most farmers living in the affected areas. Over the past several years, it has been shown that RVFV components can be expressed in baculovirus expression systems; however, this technique can only produce analytical quantities of recombinant proteins, and cannot be scaled-up for low-cost/high volume production. Here, we present the expression of recombinant RVFV proteins using baculovirally infected Trichoplusia ni insect larvae. Of the six different proteins that make up the virion of RVFV, we attempted to express the four most immunogenic components: the nucleoprotein (N), the non-structural protein (Nsm), as well as both glycoproteins (Gn and Gc). In all four cases, the resulting recombinant, soluble protein was able to produced; however, in the case of the glycoproteins, significant degredation was observed. In order to address this problem, the ectodomains of both glycoproteins were expressed. Their ability to be recognized by RVFV-infected serum strongly indicates that at least some of their native epitopes have been conserved, thereby demonstrating that these proteins have the potential for use as vaccine candidates.

Using this system, we have been able to produce quantities as large as several milligrams from the infection of a single larvae. To our knowledge, this is the first time that recombinant proteins from a bunyavirus have been expressed using this system. Furthermore, we have been able to generate significant quantities of viral glycoproteins, which have been previously shown to induce the production of neutralizing antibodies. Therefore, we anticipate that this technique can be used for the largescale production of RVFV proteins for their subsequent use in subunit vaccines.

# POSTER: ARBOVIRAL SURVEILLANCE PROGRAM ON MOSQUITOES FROM "VALLI DI COMACCHIO" AND "PARCO LOMBARDO DELLA VALLE DEL TICINO" (NORTHERN ITALY)

CALZOLARI, MATTIA [1]; BONILAURI, PAOLO[1]; BELLINI, ROMEO [2]; VERONESI, RODOLFO [2]; PILANI, ROBERTO [2]; DEFILIPPO, FRANCESCO [1]; CAIMI, MARCO [3]; PARCO, VALENTINA [4]; FEDELI, PAOLO [5]; BARBIERI, ILARIA [1]; MAIOLI, GIULIA [1]; LELLI, DAVIDE [1]; LAVAZZA, ANTONIO [1]; CORDIOLI, PAOLO [1]; DOTTORI, MICHELE [1]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL' EMILIA ROMAGNA (IZSLER)[1]; CENTRO AGRICOLTURA E AMBIENTE GIORGIO NICOLI SRL[2]; VERDEBLU SRL[3]; CONSORZIO PARCO LOMBARDO DELLA VALLE DEL TICINO[4]; DIPSA[5]

Key words: arboviruses, mosquitoes, Flavivirus, Alphavirus and Bunyavirus

Background Recently Italy was involved in two important outbreaks of human arbovirus diseases (chikungunya and West Nile). In 2008 a preliminary surveillance program in two Italian wetlands to check the presence of arboviruses in mosquitoes was activated. In this abstract the preliminary results of this program are presented.
Methods Mosquitoes were collected with $CO_2$ traps in areas with high density of mosquitoes near Comacchio (Emilia-Romagna region) and in the Ticino River Park (Lombardia region) in the mosquito season (July-October). Mosquitoes were pooled according to date, location and species. For simultaneous detection of arbovirus causing most important human and animal disease the pools obtained were tested with 3 screening PCR for the Flavivirus, Alphavirus and Bunyavirus genus. The amplified fragments were sequenced and analyzed using the Mega 4 program. Virus isolation was carried out on the same pools analyzed with PCR by cell culture (Vero and C6/C36 cells) and embryonated eggs.
Results We tested a total of 31.861 mosquitoes (369 pools), 16.156 (203 pools) from Comacchio and 15.705 (166 pools) from the Ticino River Park. The mosquitoes tested belong
to the species Anopheles maculipennis, Aedes vexans, Ae cinereus, Ae albopictus, Ochlerotatus caspius, O geniculatus, O detrirtus, Culex pipiens and Cx modestus. The most abundant species were Cx pipiens (43,2%), O caspius (25,9%), Ae vexans (13,3%), An maculipennis (2,8%). 2 pools of O caspius, captured near Comacchio on July 23rd resulted positive for the presence of Flavivirus RNA in screening PCR, 1 pool of O caspius captured in Lido di Spina (near Comacchio), on July 25th and 1 pool of An maculipennis captured in Ticino Park on August 22nd resulted positive in the Bunyavirus screening PCR. The isolation of viruses produced negative results in all the samples tested. In BLAST analysis the sequence of the positive amplified fragment from Ticino Park showed maximum homology (95%) with Batai virus (GB:AB257762). The sequence of Bunyavirus from Lido di Spina showed maximum homology (99%) with Marituba virus (GB:AY613923); it also showed a good homology (97%) with Tahyna virus (GB:U47142). The 2 pools of O. caspius positive for Flavivirus showed light similarity (less than 80%) with Cx Flavivirus (CuFV) isolated from Cx pipiens in Japan.
Conclusion Bunyavirus positive PCR found and sequenced in An maculipennis captured in Ticino Park is probably to ascribe to the presence of a Batai virus, first isolated in 1960 from this species of mosquito and not associated with human disease. The sequence of Bunyavirus obtained form Lido di Spina shows a very high homology with a virus (Marituba virus) first isolated in Amazon region in Brazil in the 60s. The second highest homology obtained in BLAST analysis is with Tahyna, a virus mainly isolated from Aedes and Ochlerotatus species that caused influenza-like symptoms in humans in Europe. The isolation of the virus failed, therefore a final classification of this positive PCR was impossible; however the presence of Tahyna virus is more probable than the presence of Marituba virus in pooled mosquitoes. The detected flaviviruses probably belong to a group of virus that are present only in mosquitoes species, so this virus does not represent a risk for human and animal populations. These positive PCR detections demonstrate that a wide range of arboviruses causing human and animal diseases could be detected by our surveillance program even in the absence of human or animal disease outbreaks.

# POSTER: APPLICATION OF REAL TIME RT-PCR METHOD IN THE DIAGNOSIS AND SURVEILLANCE OF AVIAN INFLUENZA IN POLAND

SMIETANKA, KRZYSZTOF [1]; DOMANSKA-BLICHARZ, KATARZYNA[1]; MINTA, ZENON[1]; WYROSTEK, KRZYSZTOF[1]; MORGUT, MALGORZATA [1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: avian influenza, real-time RT-PCR

Real time RT-PCR (rRT-PCR) method has been successfully used as a diagnostic tool for the detection of avian influenza (AI) virus in numerous countries of the world. In Poland, the method was applied for the first time during the outbreaks of highly pathogenic avian influenza (hpAI) caused by H5N1 subtype in wild birds in 2006. With time, it has replaced the conventional RT-PCR, which is now used only for confirmation of rRT-PCR positive results and amplification of PCR product for sequencing.

The first major validation study of the method was performed on the samples of tracheal and cloacal swabs collected from a flock of 116 mute swans in which hp AIV/H5N1 was detected in 2006. In 2007, rRT-PCR method was the major diagnostic tool during the outbreaks of hpAI H5N1 in poultry. The rRT-PCR has also been used as a screening method in the national AI survey programme and in two EU research projects and so far more than 6000 samples from poultry and wild birds have been tested.

The standard diagnostic strategy involves rRT-PCR/M for screening, and in case of positive results – rRT-PCR/H5&H7. All H5 positive samples are also tested with N1 specific primers and probes. This strategy changed during the epidemic of highly pathogenic avian influenza caused by H5N1 in 2007. After the confirmation of the first outbreak, the subsequent suspected cases were submitted for testing by rRT-PCR/H5 &N1 as a "first line" followed by rRT-PCR/M. The correlation between real time RT-PCR and conventional RT-PCR is generally high. However, there are some discrepancies between the results of rRT-PCR and virus isolation on SPF embryonated eggs with significantly higher detection rate in case of rRT-PCR. The reason for this inconsistency may be either low viral load in the samples (Ct values ususally above 30) or improper transport conditions.

The following subtypes have been identified in 2006-2008 using classical and molecular methods: H1N1, H3N8, H4N6, H5N1 (hpAIV), H5N? (lpAIV), H7N1, H7N7 (lpAIV), H13N? and numerous unidentified isolates, positive only by rRT-PCR/M. The performance of the laboratory was successfully verified in the interlaboratory proficiency tests organized by Community Reference Laboratory for AI.

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# POSTER: DEVELOPMENT OF OVINE CELL LINE DERIVED FROM PERIPHERAL BLOOD MONOCYTES AND ITS POTENTIAL USE AS IN VITRO MODEL TO STUDY THE INFECTION BY VIRUS AFFECTING RUMINANTS.

LORENZO, GEMA[1]; ORTEGO, JAVIER[1]; BORREGO, BELÉN[1]; HEVIA, ESTHER[1]; MARTIN, RAQUEL[1]; BRUN, ALEJANDRO [1]

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[1]

Key words: ovine cell line, macrophages, virus susceptibility, RNA viruses

Emerging and re-emerging transboundary animal diseases as foot and mouth disease, bluetongue and Rift Valley fever infect domestic and wild ruminants and cause high losses all over the world. Therefore, there is a need for therapies that provide rapid protection against these viral diseases.

Due to climatic changes and increasing migration, tourism and trade, exotic infectious diseases transmitted by arthropods form an increasing threat to Europe, especially since effective prevention and intervention strategies, like vaccines, are mostly lacking. A recent example is the introduction of bluetongue virus in ruminants in the Netherlands in 2006, causing large economic losses among livestock owners. Particularly worrying are viruses that not only affect livestock but also humans (so-called zoonotic viruses as is the case of Rift Valley fever virus (RVFV, Phlebovirus genus). RVFV is transmitted by mosquitoes and causes serious, often fatal disease in livestock, and it has also been known to affect humans in the form of haemorrhagic fevers of fatal consequences. The disease is endemic in Sub-Saharan Africa, although its capacity to propagate to more northern latitudes is a proven fact. There are no specific treatments against this disease, although symptomatic treatments in serious human cases exist.

It is necessary to understand the mechanisms pathogen-host interactions against viral diseases to design new vaccines that are more effective and safe. To carry out these studies we have generated an ovine cell line to simplify the manipulation of both virus and large animals. Such cells can facilitate in vitro studies of pathogen-monocyte interactions, and can supply high amounts of cells in order to carry out subsequent experiments.

In this work, we have established an ovine cell line called GeLo, and we have studied its susceptibility to be infected by three viruses (Rift Valley fever virus, bluetongue virus and foot and mouth disease virus) that infect sheep. We have generated a cell line derived from peripheral blood monocytes. The results obtained showed that the cells have the phenotypic and morphological characteristics of macrophages. RVFV and BTV were able to infect GeLo cells, and showed a similar kinetic growth at the time points studied. In addition, the virus titer obtained in GeLo cells with these two viruses was similar to BHK-21. GeLo cells were also infected by FMDV although the infection was slower and the titer was lower when compared to the infection of IBRS cells. This ovine cell line could be a useful tool in analyzing gene expression patterns following infections with pathogens affecting ruminants.

# POSTER: PRELIMINARY VALIDATION OF THE ID SCREEN BLUETONGUE EARLY DETECTION ELISA

POURQUIER, PHILIPPE[1]; LESCEU, STEPHANIE[1]

IDVET[1]

Key words: BTV immunodiagnostic early detection

1. Introduction

Since 2006, the BTV virus has spread throughout Western Europe. The widely-used VP7 competitive ELISA detects naturally-infected animals about 7 days post infection. Vaccinated animals, however, are less stimulated immunologically by the virus and seroconversion occurs more slowly.

In this context, ID VET has developed the ID Screen Bluetongue Early Detection ELISA which detects antibodies earlier in vaccinated animals than would the standard competitive VP7 ELISA. The test makes use of the presence of two Fab (on IgG) or ten Fab (on IgM) on immunoglobulins. A first Fab binds the immunoglobulins to the microplate and the other Fab binds a peroxidase antigen used as conjugate. This method is particularly suitable for the detection of IgM antibodies and therefore allows earlier testing of infected or vaccinated animals. It is at least as good as other classical tests for late immunological response.

2. Test principle

Samples to be tested and controls are added to microwells coated with VP7 protein. Anti-VP7 antibodies, if present, form an antibody-antigen complex. After incubation of a VP7 antigen-peroxidase (Po), wells are washed and the substrate is added.

3.Material and Methods

The ID Screen® BTV Early Detection ELISA was used according to the manufacturer's instructions for the analysis of:

- 6 sheep, vaccinated with the Merial BTV2 vaccine, and tested 6 days post-vaccination
- 1 goat serum from an experimental infection using the live South African BTV2 vaccine
- Field sera from 40 sheep and 50 cattle, vaccinated by the Merial BTV8 and Intervet BTV8 vaccines, respectively, and tested 24 and 50 days post-vaccination
- 302 sera from disease-free populations (Hérault, France - 2005)
- 154 sera from naturally-infected populations (Belgium - 2007)
- A pool of positive BTV1 sera collected in the South of France in the summer of 2008 and a pool of positive BTV8 sera collected in Belgium in the summer of 2007.

All samples were tested by the Early Detection ELISA as well as the conventional competitive VP7 ELISA.

3.Results

- All 6 vaccinated BTV2 sheep were found positive 6 days post-vaccination by the Early Detection ELISA, and negative by the conventional VP7 ELISA.
- The BTV2 goat serum from the experimental infection was detected 7 days post-infection by the Early Detection ELISA, and 15 days post-infection by the conventional VP7 ELISA.
- All vaccinated field sera were found positive by the Early Detection ELISA 24 days post-infection. When tested with the conventional ELISA, 31% of these sera were seronegative, 35% were seropositive, and 33% gave S/N values close to the cut-off.
- All sera tested from disease-free populations were found negative by both tests.
- All sera from naturally-infected populations were found positive by both tests.
- The Early Detection ELISA detected 2 additional dilutions of the BTV1 and BTV8 positive serum pools than did the conventional VP7 ELISA.

4.Discussion and Conclusions

These preliminary results demonstrate that the ID Screen Bluetongue Early Detection ELISA detects vaccinated animals earlier than does the conventional competitive VP7 ELISA (6-7 days post-vaccination compared with 20-40 days post-vaccination, respectively.)

Both tests, however, work equally well on naturally-infected populations and on vaccinated populations once seroconversion is complete.

# POSTER: SEROLOGICAL METHODS IN DIAGNOSIS OF POSTWEANING MULTISYSTEMIC WASTING SYNDROME

PODGORSKA, KATARZYNA[1]; STADEJEK, TOMASZ[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: PCV2, PMWS, diagnosis

Introduction

Porcine circovirus type 2 (PCV2) is main etiological agent of an emerging pig disease described as postweaning multisystemic wasting syndrome (PMWS). Epidemiological analysis indicated that PMWS spread in epizootic manner in some European countries. The virus is ubiquitous and single cases of the disease can be observed in farms without PCV2-related health problems. Therefore, the significance of the disease should be always evaluated at a herd level. Current diagnosis is based on detecting PMWS in individual pigs by histopathological examination and in situ methods combined with analysis of production parameters (1, 2).
The aim of the study is to determine serological methods for diagnosis of PMWS at a herd level.

Material and Methods

In farm No 1 blood samples were collected from 7 PMWS-affected pigs and 8 clinically healthy pigs at 10 weeks of age. Sera samples were examined by Real Time PCR to confirm PCV2 infection. Additionally cross-sectional study was performed in 4 farrow-to-finish farms affected with PMWS (Nos. 1-4) and 6 control herds (Nos. 5-10). Blood samples were randomly collected from 10-15 pigs of 5 different age groups starting from the 1st to 11th (or 13th) week of age. Antibodies specific to PCV2 were detected with in house IPMA in all sera 10-fold diluted from 1:200 to 1:102.400. Titres were expressed as logarithms.

Results

Viral DNA was present in all samples tested with Real Time PCR. In the group of affected pigs 2,8 x 109 – 2,9 x 106 DNA copies/ml (average 6,80 x 108) were detected. Examination of sera collected from control animals indicated lower level of viremia corresponding to 8,25 x 106 to 7,5 x 103 DNA copies/ml (average 1,73 x 106). In four pigs with most severe histopathological lesions of PMWS and the highest level of viremia antibodies specific to PCV2 were not detected. Only 2 diseased pigs had seroconverted with titres 3.5 and 4.7. Titres obtained for sera of clinically healthy pigs were generally higher ranging from 2.9 to 5.3.
Analysis of serological profiles revealed gradual decrease of initial maternal immunity followed by seroconversion between 5 and 9 week of age in PMWS-affected herds and between 5 and 13 week of age in control ones. The dynamic of seroconversion was different in affected and control farms. The difference was most obvious about 4 weeks post inferred infection. At this time point mean antibody titres in sera collected in affected farms gained 2.9-3.48 (mean 3.28) compared to 4.16-4.75 (mean 4.39) in healthy farms.

Discussion

Analysis of serological profile in farm No 1 indicated that pigs become infected about 5th week of age. Previous experimental infection studies indicated that specific antibodies appear about 14 dpi with PCV2 (1). However, 6 PMWS-affected pigs harboring very high virus load did not seroconvert until 10 weeks of age. Most probably it was a result of immunosuppression and an impairment of the humoral immune response described before as a feature of PMWS (2). Our results indicated that this effect was visible at the herd level. Seroconversion in PMWS-affected herds was delayed compared to control ones. This suggests that serological methods and analysis of serological profiles can be helpful in diagnosis of PMWS.

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# POSTER: BHK CELL CULTURE DERIVED FMD VIRUS PURIFICATION BY CONTINUOUS CHROMATOGRAPHY

OZDURAL, NILGUN[1]; CENGIZ, MELAHAT[1]; AKYUZ, SEREF[1]

FOOT AND MOUTH DISEASE INSTITUTE (SAP)[1]

Key words: FMD viral vaccine; hcDNA removal; continuous annular chromatography.

Recent decades have witnessed a large increase in the technological strategies for vaccine purification. Veterinary vaccines are also experiencing an upsurge in interest, especially for major problem areas such as foot and mouth disease (FMD), which is a devastating disease of live stock. Surprisingly, despite the importance of cellular DNA clearance for vaccine products, there is a paucity of literature. It was determined that tangential filtration was not efficient in removing host cell DNA from virus, despite that it is highly competent in regard of other proteins. Virus purification has traditionally been carried out by CsCl density gradient ultracentrifugation, but scale-up of this method is not practical, so it cannot meet the rising demand for purified viruses. For large scale separation and purification applications, the major drawback of preparative column chromatography is its discontinuous nature of operation. The continuous annular chromatography (CAC) is a potential and promising downstream process that allows large-scale continuous preparative chromatographic separation and purification. In CAC the resin phase is packed between the two slowly rotating concentric cylinders building up an annulus. Mobile phase are continuously loaded onto the resin through separate feed and eluent inlets that are located at the top of the resin phase. Keeping the feed and eluent inlet positions fixed, each solute in the feed is forced to flow down through the annulus. The exit angle of individual components are determined by the feed and eluent linear velocities, inlet concentrations, annulus bed height, rotating speed, mobile and stationary phase interaction. Different zones will form helices of characteristic angle which can be collected at various fixed locations around the bottom of the apparatus Here, we investigated the viability of the addition of a CAC unit as a final downstream step to the FMD virus purification process. CAC apparatus is filled with Q Sepharose XL virus licensed (GE Healthcare Bio-Sciences, Sweden) ion exchange adsorbent, where 20 mM Tris + 0.5 mM NaCl solution is used as mobile phase. During the experiments, inactivated and subsequent to several purification steps, 300K Dalton tangential flow filtered BHK cell culture virus harvest, is used as feed. The assay was calibrated with lambda DNA standard, provided in the Quant-iT PicoGreen dsDNA kit in the linear range between 1 and 1000 ng/ml using the 200-fold TE-buffer diluted reagent (PicoGreen working solution) and TE buffer for dilution purposes. For the determination of dsDNA in adjuvant free FMD vaccine, to 100 µl sample solution, 900 µL TE buffer and 1000 µL of the 200-fold TE-buffer diluted PicoGreen working solution were added respectively and incubated at room temperature for 5 min. Afterwards the fluorescence (excitation: 475 nm, emission: 515 nm) intensity was measured using a Fluorometer (Turner Designs Model 13095-02 USA). Our study provides the feasibility of continuous annular chromatographic separation of hcDNA from BHK cell culture derived virus vaccines.

# POSTER: ON-SITE DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS USING A PORTABLE, AUTOMATED SAMPLE PREPARATION AND PCR SYSTEM

REID, SCOTT[1]; MISTRY, ROHIT[2]; PIERCE, KENNETH[3]; BHARYA, SUKVINDER[2]; DUKES, JULIET[1]; VOLPE, CARMELO[2]; WANGH, LAWRENCE[3]; KING, DONALD[1]

INSTITUTE FOR ANIMAL HEALTH (IAH)[1]; SMITHS DETECTION DIAGNOSTICS[2]; BRANDEIS UNIVERSITY[3]

Key words: foot and mouth disease, LATE RT-PCR, assymetric PCR, real-time RT-PCR, Diagnosis

Introduction

Foot-and-mouth disease (FMD) is a highly contagious and economically devastating disease of farm livestock. The etiological agent, FMD virus (FMDV), is a single-stranded, positive-sense RNA virus belonging to the genus Aphthovirus within the family Picornaviridae. Rapid and accurate confirmation of the presence of FMDV is needed for effective control and eradication of the disease. An in-field detection test would be highly beneficial given that the time taken to transport suspect clinical material to a central laboratory can often be lengthy, thus delaying confirmation of infection in the event of an outbreak. This study describes the development of a molecular assay for the detection of all seven serotypes of FMDV using novel technology, namely: Linear-After-The-Exponential (LATE)-PCR [1], for use in a portable, easy-to-use, fully automated sample preparation and PCR instrument.

Materials and Methods

Primers and a mismatch tolerant probe were designed from consensus sequences in the FMDV 3D (RNA polymerase) gene to detect the target and its variants at low temperature. An internal control (IC) was included to validate negative results. After demonstrating that the LATE RT-PCR signal at end-point was proportional to number of target molecules over the range 10 to 1 million copies, the assay was compared with a one-step real-time RT-PCR (rRT-PCR) assay (targeting the 3D) used routinely by reference laboratories [2].

Results

The LATE RT-PCR assay amplified RNA extracted from multiple strains of all FMDV serotypes. Of the 121 FMDV positive samples tested, 118 were positive by virus isolation and 119 were positive by both rRT-PCR and LATE RT-PCR tests. Twenty-eight FMDV negative samples failed to react in all 3 tests. There were no false positive signals with RNA from other vesicular disease-causing viruses. Each FMDV-negative sample generated a signal from the IC, ruling out amplification failures. A dilution series of an FMDV reference strain demonstrated that the analytical sensitivity of the assays was similar.

Discussion and Conclusions

The LATE RT-PCR assay is at least as informative as the current rRT-PCR assay used in reference laboratories. All the reagents required to perform the assay have been converted into ready-to-use dry-format. The FMDV assay reagent cartridge, in conjunction with the sample preparation unit and the BioSeeqTM-Vet Portable Diagnostic Laboratory [3] is a fully integrated, easy-to-use system, which is currently undergoing field evaluation.

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# POSTER: REVIEW ON RING TRIALS ORGANISED WITHIN EPIZONE AND PERSPECTIVES FOR STANDARDISATION

GRILLET, COLETTE[1]; SIONNEAU, DOMINIQUE[1]; ALBINA, EMMANUEL[1]

CENTRE DE COOPÉRATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT (CIRAD)[1]

Key words: ring trials, quality assurance, standards, diagnostics

EPIZONE is a network of excellence of European or national reference laboratories. Its purpose is to integrate researches in the field of epizootic diseases in order to improve the scientific excellence and avoid duplication. A theme of EPIZONE (theme 4) is dedicated to the development and standardisation of the diagnostic tools for epizootic diseases. To achieve this objective, regular ring trials are organised within the network. However, to our knowledge, they are designed and organised by different laboratories with no established standards. A recent review of the first European Networks of Excellence pointed out that the main collective goods produced by successful networks "Definitions, measurement protocols, procedures and quality assurance" and "standards and definitions of specifications related to products or technologies" (1). Quality assurance is also a pressing need for laboratories producing reference materials or delivering diagnostic results in the field of epizootic diseases (often notifiable diseases!). We believe that EPIZONE could somehow play a role in improving quality, particularly in the present case, for the standardisation of ring trials designed for the comparison or validation of diagnostic methods. As a starting point, we decided to review three European ring trials into which we took part (AIV/RRT-PCR, BT/ELISA & PCR(2), ASF/ELISA & PCR), one that we are currently organising for EPIZONE (PPR/ELISA & PCR) and three others that we have organised in 2008 either at the international, European or national level (AIV/RRT-PCR, Culicoides/PCR, BT/ELISA). The number of participants in these ring trials varied from 5 to more than 80 with an average of 20-30. The number of reference materials included in the trials ranged from 8 to 38, with half of the trials using just 10 samples. All trials included positive and negative samples. However, only 3 and 1 (none from EPIZONE), included a serial dilution of materials and repeated samples, respectively. Four and five trials included mock samples and random codes, respectively. For all trials, a final global report was delivered, but only 6 of them supplied an individual report with a statement on the results obtained by the laboratory. From this evaluation, it is possible to make suggestions for harmonisation and EPIZONE could promote the definition of guidelines for the organisation of such ring trials, taking into account the international standards available (3, 4). These guidelines should describe general and specific rules for inner standardisation with the aim to develop international acknowledgment of EPIZONE research standards.

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# POSTER: THE COMPARATIVE STUDY OF EFFECT OF SALINOMYCINE 12% ALONE, SALINOMYCINE 12% PLUS BETAINE 96% AND PARACOX 5 VACCINE ON GROWTH, PRODUCTIVE PARAMETERS AND AGAINST COMMON EIMERIA IN BROILER

ZAKERI, AFSHIN[1]; FADAEI, MARYAM[2]; MIRZA AHMADI, SINA[3]

ISLAMIC AZAD UNIVERSITY, TABRIZ[1]; TARBIAT MODARES UNIVERSITY[2]; ISLAMIC AZAD UNIVERSITY, ZANJAN[3]

Key words: coccidiosis, productive parameters, paracox 5, salinomycine, betaein

The purpose of this research is studying about efficacy of betaine ( the effect of this drug for the functional effect of salinomycine) , ionophores drugs and coccidiosis vaccine on growth parameters such as weight gain, feed consumption ratio and against common eimeria in broiler chickens. In this study 480 Cobb 500 broiler chickens were divided in four similar groups with 120 chickens in each group (with four replicates of 30 chickens in each group). 0.5 kg / ton salinomycine 12% in , 1.5 Kg/ ton betaine 96% + 0.5 kg / ton salinomycine 12%Iexperimental group III and 0.48 cc paracox5 vaccine in experimental group IIin experimental group were added to the basic diets however the control group were fed only with basic diet . Every week 40 chickens from each group selected randomly and productive parameters such as weight gain, FCR, EEF, mortality , feed intake were calculated. At 21 day, all of the chickens were contaminated experimentally through oral by mixed of four species of common Eimeria in Iran (Eimeria acervulina, E.tenella, E.maxima and E.necatrix). From the seventh day after contamination, their faeces were collected and have been done OPG separately. OPG significantly ($P < 0.05$) between each four groups were obtained , final FCR was significant( PIIexperimental group $< 0.05$ ) but others productive parameters were not significant..Generally using 0.15%( 1.5 Kg / ton) of betaine 96%) can improve productive factors and performance of coccidiostatic drugs, also using of coccidiosis vaccines were so useful for perevention of coccidiosis.

# POSTER: CROSSPROTECTION BETWEEN WEST NILE VIRUS LINEAGES

SOTELO, ELENA[1]; LLORENTE, FRANCISCO[1]; JIMENEZ-CLAVERO, MIGUEL ANGEL[1]

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[1]

Key words: crossprotection, lineages, mice, west nile virus

West Nile virus (WNV) is an arthropod-borne flavivirus belonging to the Japanese Encephalitis serogroup, having transmission cycles involving birds as amplifying hosts, and zoonotic potential. In the Western Mediterranean region, WNV is an old known virus, causing sporadic outbreaks interceded by long periods of epidemiological silence, however in North America the virus has spread relentlessly, causing a severe epidemic of disastrous consequences for public health, livestock and wildlife.

Intraperitoneal injection of WNV into mice generally leads to fatal encephalitis which has been proposed as an useful model for human encephalitic disease. Neurovirulence of WNV in laboratory mice has also been reported to show some virus strain-to-strain variation.

In Europe there is recent evidence of circulation of WNV different lineages, likely differing in virulence. On the other hand, it is well known that the antibodies to different WNV lineages cross-neutralize. Thus it is possible that pre-existing immunity to one low-virulence WNV lineage in European (avian) reservoir populations could be protective against more virulent strains, preventing their spread. In order to test this hypothesis we compared the different neurovirulence in mice between two representative strains of the most ubiquitous linages 1 and 2 of WNV and find out if there is any crossprotection between them.

Groups of 4 weeks old female Swiss mice were inoculated intraperitoneally with doses ranging from 1 to 1000 PFU of WNV New York 1999 (NY99) and Uganda 1937 (B956) belonging to WNV lineage 1 and 2 respectively. Daily observation of the mice health status was carried out, registering neurological signs. Deaths were recorded from day 7 to day 14 after inoculation. Brain samples were recovered from the dead individuals and analyzed by PCR and virus isolation. While there were survivors in every group inoculated with B956, the whole group inoculated with 100 PFU of NY99 died. Average survival time was very similar for both viruses. However lethal doses were considerably different in the two isolates: 2.31 for NY99 and 39.32 for B956.

On the day 27 after the first inoculation, survivors of the first inoculation with B956 were challenged with a lethal dose of NY99 (100 PFU). Similarly to the first experiment, deaths took place between days 7 and 13. Monitoring of the health status and sample collection were done in the same manner as in the first part of the experiment. While between the groups of mice previously inoculated with low doses of B956 (1 and 10 PFU) we registered several cases of mortality, all the mice previously inoculated with the higher doses (100 and 1000 PFU) survived the second challenge with NY99. In conclusion, subclinical doses of WNV B956 (Lineage 2) crossprotected against a lethal dose of WNV NY99 (Lineage 1).

Further studies are on-going to characterize this observed effect of immunity protection between WNV lineages 1 and 2 and to find out the mechanisms that are involved in these reactions.

# POSTER: MONOCLONAL ANTIBODIES RAISED AGAINST RVFV NUCLEOCAPSID PROTEIN FOR DEVELOPMENT OF COMPETITIVE SERODIAGNOSTIC ASSAYS

MARTÍN, RAQUEL[1]; LORENZO, GEMA[1]; BOSHRA, HANI[1]; BORREGO, BELÉN[1]; BRUN, ALEJANDRO[1]

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[1]

Key words: Rift Valley fever, nucleoprotein N mAbs, competition ELISA

This work describes the development of monoclonal antibodies (mAbs) directed to immunodominant nucleoprotein N epitopes of Rift Valley fever virus (RVFV), and their subsequent use in a competitive ELISA for the detection of serum anti-RVFV antibodies. Mabs specific for the nucleocapsid (N) protein of the virus were generated after DNA immunization of Balb/c mice and characterized by Western blot, ELISA and immunohistochemistry. Several mAbs were able to compete for the binding of anti-RVFV serum to nucleoprotein N. Competition of mAbA9F12 with all mAbs generated allowed the identification of at least two different immunorelevant epitopes. Moreover the use of mab A9F12 in a sandwich assay either as a capture or detector antibody was tested. These mAbs constitute excellent tools for the detection of RVFV specific antigen as well as for the specific identification of serum RVFV antibodies in different species.

# POSTER: A MOLECULAR EPIDEMIOLOGY TOOL FOR GENOTYPING OF RVFV ISOLATES

IGLESIAS, JAVIER[1]; HEVIA, ESTHER[1]; BRUN, ALEJANDRO[1]

CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[1]

Key words: Rift Valley fever, M segment, genetic lineages,

RVFV is a mosquito borne virus causing recurrent outbreaks of disease in humans and livestock in Africa. Recently several outbreaks have occurred in Kenya and neighbouring countries, as well as in Madagascar. The spreading ability of this virus is documented since several outbreaks have occurred in other countries far away from tropical Africa such as Egypt, Yemen and Saudi Arabia. A recent work by Bird and colleagues (J. Virol 81, 2805-16) generated the sequence of 33 diverse RVFV isolates ecologically and biologically diverse and their genetic relationship was established. Although the global viral diversity was low (aprox 5%) a significant nucleotide variation was found mainly associated to the carboxy terminus of Gn (G2), the amino terminus of Gc (G1) and at the 3′ UTR.

Aimed to identify a quick method to establish genetic lineages among RVFV isolates we focused in one of the RVFV genome regions where maximal divergence at the nucleotide sequence was found. This region comprised a fragment of 480 nt (nts 3405 to 3885) at the 3′ end of the M segment who was subjected to phylogenetic analysis using the Neighbor-Joining/UPGMA method (version 3.6a2.1) using the Bioedit Program. Up to date, 68 sequences available in the GenBank were used for this analysis. The results showed a strong correlation to those obtained using the complete M segment sequences, and make it possible to unequivocally identify a RVFV strain on the basis of its 3405-3885 amplicon sequence; thus indicating the reliability of the method.

Finally amplicons corresponding to the 3405-3885 region were generated by RT-PCR of four South African RVFV isolates (AR20368; AN1830; 252/75 and 54/74), sequenced and a phylogenetic analysis was performed as described above.

# POSTER: RESULTS FROM THE 2008-2009 PROFICIENCY TESTING SCHEME FOR REAL TIME RT-PCR METHODS USED TO DETECT NEWCASTLE DISEASE VIRUS (APMV-1)

VAN BORM, STEVEN[1]; ALDOUS, ELIZABETH[2]; GRUND, CHRISTIAN[3]; OBISHAKIN, EMMANUEL[1]; FULLER, CHAD[2]; KOCH, GUUS[4]; JESTIN, VÉRONIQUE[5]; CHERBONNEL, M[5]; HANDBERG, KURT[6]; STAHL, KARL [7]; THORÉN, PETER[7]; FERNANDEZ-PINERO, JOVITA[8]; CATTOLI, GIOVANNI[9]; SMIETANKA, KRZYSZTOF[10]; ALBINA, EMMANUEL[11]; COVEN, FETHIYE[1]; ALEXANDERSEN, SOREN[12]; PASICK, JOHN[12]; HOFFMANN, BERND[3]

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE (VAR)[1]; VETERINARY LABORATORIES AGENCY (VLA)[2]; FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[3]; CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[4]; AGENCE FRANÇAISE SÉCURITÉ SANITAIRE DES ALIMENTS (AFSSA)[5]; NATIONAL VETERINARY INSTITUTE DTU (VET-DTU)[6]; NATIONAL VETERINARY INSTITUTE (SVA)[7]; CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[8]; INSTITUTO ZOOPROFILATTICO SPERIMENTALE VENEZIE[9]; NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[10]; CENTRE DE COOPÉRATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT (CIRAD)[11]; CANADIAN FOOD INSPECTION AGENCY[12]

Key words: Real time RT-PCR, Newcastle disease, ring trial, APMV1

1. Introduction and objectives: this poster describes the results from the inter-laboratory proficiency testing scheme conducted in late 2008-beginning 2009 to evaluate real time RT-PCR assays used to detect Newcastle disease virus (NDV). This study was organised in the framework of EPIZONE WP4.1 and focuses on detection of viral RNA by real time RT-PCR

2. Material and methods: a panel of 26 coded RNA samples was prepared and send to 13 participating labs. RNA was purified from diluted virus stocks and stabilized in RNA safe buffer (Hoffmann, et al., 2006). Each laboratory was asked to apply its method(s) of choice for the detection of NDV RNA.

3. Results: A variety of real time RT-PCR protocols was used, targeting the Matrix gene (M; 10/13 labs), Large Protein gene (L; 6/13 labs), Fusion Protein gene (F; 3/13 labs), and Nucleoprotein gene (NP; 1/13 labs). Dilution studies indicated that these assays differed in analytical sensitivity by as much as 1000 fold. Overall the best sensitivity was demonstrated using the M and L gene (down to 100 $EID_{50}$/ml of sample before RNA extraction, respectively). The lowest sensitivity was observed using protocols targeting the Fusion protein gene. The panel also included a diversity of isolates, covering as much of the phylogenetic diversity of APMV1 viruses (both class I and class II isolates; Kim, et al., 2007) as could be obtained. A deficiency of most assays in the detection of class I isolates was demonstrated. Most M gene targeted tests seem to be able to detect the full diversity of Class II isolates but fail for Class I isolates. Fortunately, complementary tests were identified (L gene or NP gene targeted) that successfully detect Class I isolates.

4. Discussion: Large differences between tests were observed. These results provide test users with solid data to interpret whether a test is fit for purpose. Candidate tests for European standardisation could be identified.

5. Acknowledgements: This work was supported by EPIZONE (WP4.1). M. Boschmans and M. Decaesstecker provided excellent technical support.

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# POSTER: SEROLOGICAL INVESTIGATION FOR CHICKUNGUNYA VIRUS IN DIFFERENT ANIMAL SPECIES REARED IN THE AREA OF ITALIAN OUTBREAK IN 2007

LELLI, DAVIDE[1]; MORENO, ANA[1]; SOZZI, ENRICA[1]; CANELLI, ELENA[1]; TAMBA, MARCO[1]; CAPUCCI, LORENZO[1]; BROCCHI, EMILIANA[1]; CORDIOLI, PAOLO[1]

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLA LOMBARDIA E DELL' EMILIA ROMAGNA (IZSLER)[1]

Key words: Chikungunya, monoclonal antibodies, competitive ELISA, serology, surveillance

Introduction

The Chikungunya fever epidemic occurred in summer 2007 in Italy represents the first autochthonous European outbreak of a tropical disease transmitted by vectors (1,2).

In this study Chikungunya virus (CHIKV) isolated in Emilia Romagna (Italy) was used for the production of monoclonal antibodies (MAbs) subsequently used in a competitive ELISA test for anti-CHIKV antibodies detection in animal sera belonging to different species reared in the area of CHIKV outbreak.

Material and methods

Virus. The virus used for the MAbs production and as antigen in the ELISA test is the strain 209395/07 isolated by the laboratory of IZSLER from an homogenized of insect pool (Aedes albopictus) that was previously resulted positive by PCR for CHIKV.

Monoclonal antibodies (MAbs). MAbs were produced using standard method. The screening and the following characterization were performed by indirect ELISA, immunoperoxidase (IP), virus-neutralization (VN) and western blotting (WB).

Sample sera. 20 known human sera (10 positive and 10 negative) and 493 animal sera belonging to five different species were analysed. The 493 animal sera are subdivided as following: 256 dog sera, 28 nutria sera, 79 chicken sera, 123 pigeon sera and 7 rabbit sera.

Results

Monoclonal antibodies. Forty five MAbs reactive against CHIKV were produced, 9 of these with VN activity. In addition, two anti-CHIKV MAbs (1H7 and 1E10) resulted positive in WB, revealing a band with a molecular weight of 50 Kd corresponding to both E1 and E2 glycoproteins of the envelope (3). The proteomic analysis confirmed that the protein was E2.

Considering the reactivity in the screening test (indirect ELISA and IP), the virus neutralizing activity and WB results, 2 MAbs (1H7 and 1A7) were selected, cloned and conjugate with HRP for the development of a competitive ELISA test. By competition ELISA analysis it resulted that 1H7 and 1A7 has a reciprocal competition against virus binding. In particular, they do not bind independently to the E2 protein and react respectively against a linear and a conformational epitope present on the protein.

Competitive ELISA for anti-CHIKV antibodies detection. ELISA test was developed using in parallel the 2 conjugated MAbs 1A7 and 1H7. 96 wells plate were coated with partially purified antigen, four dilution (1/5 to 1/40) of each sample sera were distributed and for a simultaneous competition with test sera the selected MAb were added in the plate. The ability of sample sera to inhibit the binding of specific MAb-conjugate to the antigen was then evaluated and results were expressed as percentage of inhibition.

Serology. All the 20 human sera were correctly identified and the 10 positive showed an elevated reactivity, The 493 sera of different animal species didn't show any reactivity, resulting negative to anti-CHIKV antibodies.

Conclusions

The serological diagnosis using a test easily applicable to different species like the competitive ELISA,could represent a valid diagnostic tool in the study of the epidemiology of CHIK and the effective role of animals in the virus spreading. These results suggest that, during the Italian epidemic, the CHIKV presumably circulated only in an urban cycle (human-vector-human).

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# POSTER: COMPARISON OF DIFFERENT DETECTION SYSTEMS FOR AVIAN INFLUENZA VIRUS DETECTION IN OROPHARYNGEAL AND CLOACAL SWABS FROM EXPERIMENTALLY INFECTED BIRDS

MINTA, ZENON[1]; SMIETANKA, KRZYSZTOF[1]; WYROSTEK, KRZYSZTOF[1]

NATIONAL VETERINARY RESEARCH INSTITUTE (NVRI)[1]

Key words: avian influenza, detection methods

For diagnostic purposes of avian influenza (AI), the selection of proper samples and test methods is of fundamental importance in order to make diagnosis rapid and reliable. Virus isolation (VI) and identification is the gold standard laboratory method but it is also time consuming. By using PCR method, results can be available within one day. Immunochromatographic tests have been developed for rapid detection of influenza A viral antigen from suitable specimens and can be used in a laboratory or as pen-site tests for screening of AI infection in poultry flocks. In the study, samples collected from chickens experimentally infected with AI virus (AIV) were used to assess sensitivity of two commercial rapid immunochromatographic assays and two nucleic acid detection tests (RT-PCR/M and real time RT-PCR/M) and comparing them to the VI test.

To assess the analitical sensitivity of the diagnostic protocols, serial 10-fold dilutions of low pathogenic AI (LPAI) virus of H7N1 subtype were made in PBS and each dilution was tested by the rapid test kits and two nucleic acid detection methods. Rapid kits could detect equivalent to 10$^5$ (kit 1) and 10$^6$ (kit 2) EID$_{50}$/1 ml unlike RT-PCR and real time RT-PCR for which detection limit was determined to be equivalent to 10$^2$ and 10 EID$_{50}$/1 ml, respectively. For the challenge experiment, groups of five SPF chickens were inoculated intraocularly and intranasally with 10$^{6,5}$ EID$_{50}$ of LPAI H7N1 virus ( Group 1) or with 10$^4$ EID$_{50}$ (Group 2) and 10$^6$ EID$_{50}$ (Group 3) of highly pathogenic AI (HPAI) isolates of the H5N1 subtype isolated from swans in Poland in 2006. Oropharyngeal and cloacal swabs were collected from each bird on days 2, 4, 7, 10, 14 and 21 (Group 1) and at 24-hr intervals p.i. (Groups 2 and 3) and suspended in 2 ml of PBS. Individual swabs and pooled (up to 5 swabs/pool) were tested in the rapid tests, RT-PCR, real time RT-PCR and virus isolation (only pooled samples). The results indicate that after infection of SPF chickens with 10$^{6,5}$ EID$_{50}$ of LPAI virus (Group 1), virus isolation was positive in the pooled tracheal swabs at days 2 and 4, and in the pooled cloacal swabs between 2 and 7 days. Molecular methods detected the viral RNA up to day 14, with most birds shedding the virus between 2-7 days p.i. with 20/60 samples and 18/60 samples positive by real time RT-PCR and RT-PCR, respectively. The rapid tests detected influenza A antigen only from cloacal swabs between day 2-7 (kit 1) and day 2–4 (kit 2) with 4/50 samples and 2/50 samples positive, respectively. All SPF chickens challenged with HPAI H5N1 died between 3-7 day (Group 2) and 2-4 day p.i.. Virus isolation was positive in the pooled oropharyngeal and cloacal swabs between 1-7 day (Group 2) and 1-3 day (Group 3). Real time RT-PCR amplified influenza RNA in swabs collected between day 2-7 (Group 2) and day 1-3 (Group 3) while RT-PCR was able to detect viral RNA from samples collected from day 1 to 7 (Group 2) and day 2-3 (Group 3) p.i. with 32/68 and 21/68 samples positive , respectively. Only 3 oropharyngeal swabs were positive by the kit 1(2 samples at day 3 and 7 in Group 2, and 1 sample at day 2 in Group 3) and 1 oropharyngeal swab was also positive by the kit 2 at day 7 p.i. in Group 2 from 68 sample tested.

The results are disappointing, as tested rapid diagnostic kits have proven to be insensitive for detection AI virus when compared with virus isolation and nucleic acid detection tests.

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# POSTER: BETWEEN-PEN TRANSMISSION OF FOOT-AND-MOUTH DISEASE VIRUS IN UNVACCINATED AND VACCINATED PIGS

VAN ROERMUND, HERMAN[1]; EBLE, PHAEDRA[1]; DE JONG, MART[2]; DEKKER, ALDO[1]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]; WAGENINGEN UNIVERSITY AND RESEARCH CENTRE[2]

Key words: FMD, pigs, transmission, reproduction ratio, vaccination

Three transmission experiments were carried out to quantify between-pen transmission of Foot-and-Mouth Disease virus (FMDV, strain O/Net/2001). The experiments consisted of two replicates of five infectious pigs housed in a central square-shaped pen which was surrounded by four separate smaller pens, each housing one naïve pig. The infectious pigs were infected before by seeder pigs. Age of the animals was ca 10 weeks. In Experiment 1 all pigs were unvaccinated and the distance between the central pen and each contact pen was 40-70 cm. The wooden pen walls were 125 cm in height and placed on the floor without cement. In Experiment 2 all pigs were unvaccinated again but the distance between the central and the contact pens was 0 cm, thus the infectious pigs were separated from each contact pig by a single pen wall. In experiment 3 all pigs were vaccinated (-14 dpi, FMD O Manisa vaccine) and the distance between the central and contact pens was 0 cm again. Of all animals OPF samples were taken daily, clinical symptoms were scored daily, and serum samples were taken twice a week during the experimental period of three weeks. OPF samples were tested for the presence of virus using a VI-assay. Serum samples were tested in a VN-assay and NS-ELISA.

In both replicates of the three experiments all five pigs in the central pen were OPF VI-positive and thus infectious during several days. In Experiment 1, none of the eight contact pigs became infected, as all tests were negative throughout the experiment. The basic reproduction ratio R0 (the average number of new infections by one infected animal in a susceptible population) was estimated as 0 (with 95% CI: 0-0.08), which is significantly below 1 ($p<0.001$). The average duration of the infectious period was 8.3 (7.7-8.9) days. In Experiment 2, respectively one and three of the four contact pigs became infected and R0 was estimated as 1.1 (0.3-2.6), which is not significantly different from 1 ($p=0.86$). The average duration of the infectious period of the infectious pigs was 7.8 (7.1-8.3) days. In Experiment 3 with vaccinated pigs, none of the contact pigs became infected and R0 was estimated as 0 (0-0.3), which is significantly below 1 ($p<0.001$). The average duration of the infectious period of the infectious (vaccinated) pigs was 4.6 (3.9-5.4) days.

The vaccine had a significant effect on the pen to adjacent pen transmission, as R0 of vaccinated pigs (from pen to adjacent pen, Experiment 3) is significantly lower than that of unvaccinated pigs (Experiment 2) ($p<0.001$). Using earlier experiments as well, we conclude that a single pen wall between adjacent pigs reduces the FMDV transmission a 20-fold compared to within-pen transmission, for both unvaccinated and for vaccinated pigs. For unvaccinated pigs however, R0 is still 1.1 (Experiment 2), but for vaccinated pigs R0 is reduced significantly below 1 (Experiment 3).

# POSTER: ESTABLISHMENT OF A REAL-TIME RT-PCR ASSAY FOR RAPID DETECTION OF BOVINE VIRAL DIARRHEA VIRUS (BVDV) AND ANALYSIS OF CATTLE SERA FOR BVDV ANTIGEN BY ELISA IN TURKEY

TURAN, NURI[1]; HASAN, SAGED[1]; YILMAZ, HUSEYIN[1]

UNIVERSITY OF ISTANBUL, VETERINARY FACULTY[1]

Key words: BVDV, real-time RT-PCR, cattle and ELISA and Turkey

Bovine viral diarrhea (BVD) is an economically important viral disease of cattle. Rapid detection and culling of the persistently infected animals is essential in the control and eradication of BVD. Therefore, this study was contucted to establish a real-time PCR assay to use for rapid detection of BVDV and to investigate the frequency of bovine viral diarrhea virus antigen in cattle blood by ELISA. The blood samples analysed in this study were originated from different regions of Turkey. 120 cows from a farm in Aegean region, 181 cows from another farm in Aegean region, 11 cows from East Anatolia, 7 cows from Black Sea region and 22 calves from Marmara region were analysed by ELISA. The presence of antigens of BVDV in sera were as follows: no positivity in 120 cows, 4 positives in 181 cows, no positivity in 11 cows, 2 positives in 7 cows and 1 positive in 22 calves, respectively acording to regions as mentioned above. 10 blood sera were also anlysed by optimised real-time RT-PCR using a SYBR Green assay and 2 of the cattle were found to be positive for the presence of BVDV-2 RNA. The bands were seen on agarose gel in positive control, positive samples but not in negative control. No positivity was found for BVDV-1 in 10 blood samples. Threshold cycle (CT ) values of the positive control was 28 while 36-37 for the positive samples. The melting curves of the positive controls and positive samples were the same (85 C). In conclusion, the real-time RT-PCR assay established in this study will help rapid detection of BVDV. The isolation and sequencing studies are going on for BVDV-2 detected in this study.

# POSTER: THE VIRAL REPLICATION COMPLEX IS ASSOCIATED WITH VIRULENCE OF NEWCASTLE DISEASE VIRUS (AVIAN PARAMYXOVIRUS TYPE 1)

DORTMANS, JOS[1]; ROTTIER, PETER[2]; KOCH, GUUS[1]; PEETERS, BEN[1]

CENTRAL VETERINARY INSTITUTE OF WAGENINGEN UR (CVI)[1]; DEPARTMENT OF INFECTIOUS DISEASES & IMMUNOLOGY[2]

Key words: Newcastle disease virus, ppmv-1, replication complex, virulence

The fusion (F) protein of Newcastle disease virus (NDV, also known as avian paramyxovirus type 1; APMV-1) is required for the initiation of infection and acts as a major determinant of virulence. Virulent NDV strains can be discriminated from low virulent strains by the presence of multiple basic amino acid residues at the proteolytic cleavage site of the F protein. However, some pigeon paramyxovirus type 1 (PPMV-1) strains are low virulent, despite the fact that their F protein cleavage site contains multiple basic amino acids. To determine the molecular basis for the low pathogenicity of these strains, we constructed infectious full-length cDNA clones of the low virulent PPMV-1 strain AV324 and the highly virulent NDV strain Herts. Using a reverse-genetics approach, genes were exchanged between these strains and the recovered chimeric viruses were evaluated for their virulence in chickens. Our results showed that the virulence of F chimeric viruses was similar to that of their respective parental viruses, indicating that the F protein of AV324 is not functionally different from that of a virulent strain. However, replacement of the replication proteins NP, P and L of AV324, either together or individually, with those of Herts significantly increased the virulence of the chimeric viruses. Since it has been shown that low virulent pigeon paramyxovirus isolates can become virulent after passage in chickens, we passaged AV324 in one-day-old chickens using intracerebral inoculation. Virus from every passage showed a gradual increase in virulence. Sequence analysis showed that two amino acids in the L protein and one amino acid in the P protein had changed after six passages. Our results strongly suggest that not the F protein but the viral replication complex consisting of the NP, P and L proteins, plays a key role in the low virulence of some PPMV-1 isolates.

# POSTER: CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF VACCINE STRAIN AND TURKISH FIELD STRAIN OF ORF VIRUSES

OGUZOGLU, TUBA CIGDEM[1]; ATALAY-VURAL, SEVIL[1]; KARAPINAR, ZEYNEP[1]; ALKAN, FERAY[1]

ANKARA UNIVERSITY VETERINARY FACULTY[1]

Key words: Contagious Ectyma/Orf Virus, Major envelope protein gene, sequences, Turkey

The ORF virus, one member of the Parapoxviridae, outbreaks have been reported annually in Turkey. The causative agent ORF viruses in Turkey has been described clinically, pathologically and in electron microscope studies for many years. In this study the field isolates of Orf viruses from a goat and 4 sheep, and also vaccine strain from sheep, were identified depend on partial sequence of major envelope protein gene (B2L). Data showed that the Orf viruses from Turkey was closer to the Indian then Iran isolates. This is the first study on the molecular characterization and phylogenetic analysis of Orf viruses in Turkey.

# POSTER: THE FEASIBILITY OF DEVELOPING A RISK ASSESSMENT FOR TICKBORNE LIVESTOCK DISEASES EMERGING IN EUROPE DUE TO CLIMATE CHANGE

GALE, PAUL[1]; MARTINEZ, MARTA[2]; WILSON, ANTHONY[3]; CAPELLI, GIOIA[4]; ULRICH, RAINER[5]; LINDBERG, ANN[6]; PHIPPS, PAUL[1]; YIN, HONG[7]; DE LA TORRE, ANA[2]; MUNOZ, MARIA JESUS[2]

VETERINARY LABORATORIES AGENCY (VLA)[1]; CENTRO DE INVESTIGACIÓN EN SANIDAD ANIMAL (CISA-INIA)[2]; INSTITUTE FOR ANIMAL HEALTH (IAH)[3]; INSTITUTO ZOOPROFILATTICO SPERIMENTALE VENEZIE[4]; FRIEDRICH-LOEFFLER-INSTITUTE (FLI)[5]; NATIONAL VETERINARY INSTITUTE (SVA)[6]; LANZHOU VETERINARY RESEARCH INSTITUE (LVRI)[7]

Key words: Climate change, risk assessment, tick-borne disease, Crimean-Congo haemorrhagic fever

1.Introduction and Objectives

Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne virus which infects livestock and causes serious illness in humans. It may be transmitted to humans through handling meat from infected livestock. It is currently emerging in Turkey and the Balkans where it is believed to be transmitted by the tick Hyalomma marginatum which favours low humidity. The objective of the work presented here was to assess the feasibility of developing a risk assessment model for the impact of climate change on the emergence of CCHFV in the European Union (EU).

2.Material and methods

A risk pathway for transmission of CCHFV to livestock and wildlife hosts was set out based on the OIE (2004) release, exposure and consequence framework. Expertise on the impact of climate change on the distribution of the tick H. marginatum and on the epidemiology of CCHFV was provided by Prof. Agustín Estrada-Peña (Zaragosa, Spain) and Dr. Valérie Mioulet (Health Protection Agency, UK), respectively.

3.Results

A geographical information system (GIS) model based on five layers was proposed. Layer 1 represented the risk of CCHFV infection in a susceptible animal given release into the EU; Layer 2 assessed the risk of exposure of indigenous ticks to those CCHFV-infected animals in Layer 1; Layer 3 represented the risk of exposure of vertebrate hosts to those infected ticks in Layer 2; Layer 4 represented the risk of exposure of livestock to those ticks in Layer 2; and Layer 5 was the suitability for transmission based on the basic reproductive ratio (R0). Layer 2 would include southern ticks, namely H. marginatum, and northern ticks, namely Ixodes ricinus.

4.Discussion and Conclusions

In conducting the feasibility study, it was noted that a single model to represent the climate impact on different populations of H. marginatum is not feasible for Layer 2. Moreover, determining R0 values for tick-borne diseases for Layer 5 is complex and potentially data intensive. It was concluded that it would be possible at this stage to begin populating the layers so that areas of overlap of the virus release, range of appropriate tick vectors and wildlife vectors could be assessed in relation to the distribution of livestock.

5.Acknowledgements

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EPIZONE

# Participant list

**A**

| | | |
|---|---|---|
| AIT-ALI | TAHAR | Roslin Institute |
| AKCAEL | ESIN | TUBITAK-MAM |
| AKSAKAL | ABDULBAKI | Yüzüncü Yil Üni. |
| AKYÜZ | ŞEREF | ŞAP Enstitüsü |
| ALBAYRAK | HARUN | Samsun VKAE |
| ALBINA | EMMANUEL | CIRAD |
| ALDOUS | ELIZABETH | VLA |
| ALEXANDERSEN | SOREN | NCFAD |
| ALKAN | MUSA | ŞAP Enstitüsü |
| AMADORI | MASSIMO | IZSLER |
| ARAT | SEZEN | TUBITAK-MAM |
| AUDONNET | JEAN-CHRISTOPHE | MERIAL |
| AUTORINO | GIAN LUCA | IZSLT |
| AYNAGOZ | GULHAN | ŞAP Enstitüsü |

**B**

| | | |
|---|---|---|
| BARNES | HELEN | Fort Dodge |
| BARROS | SILVIA | LNIV |
| BASHIRUDDIN | JOHN | IAH |
| BATTEN | CARRIE | IAH |
| BEER | MARTIN | FLI |
| BELLINI | SILVIA | IZSLER |
| BENSABER | AMINA | SEPPIC |
| BERG | MIKAEL | SVA |
| BERGMANN | SVEN M. | FLI |
| BIGARRE | LAURENT | AFSSA |
| BLANCHARD | YANNICK | AFSSA |
| BONGERS | JOHAN | CVI |
| BONILAURI | PAOLO | IZSLER |
| BOONE | IDES | VAR |
| BORREGO | BELEN | CISA-INIA |
| BOSCH LOPEZ | JAIME | CISA-INIA |
| BOSHRA | HANİ | CISA-INIA |
| BOTNER | ANETTE | VET-DTU |
| BOUET | CORALINE | AFSSA |
| BOULOY | MICHELE | Instıtut Pasteur |
| BREARD | EMMANUEL | AFSSA |
| BRIAND | FRANÇOİS-XAVİER | AFSSA |
| BRUN | ALEJANDRO | CISA-INIA |
| BRUNHART | IRIS | BVET |
| BUCZKOWSKI | HUBERT | IAH |
| BULUT | NACİ | ŞAP Enstitüsü |

**C**

| | | |
|---|---|---|
| CAGIENARD | ARIANE | MERIAL |
| CAMUS | EMMANUEL | CIRAD |
| CAPELLI | GIOIA | IZS-Ve |
| CARLSSON | ULLA | SVA |
| CAROCCI | MARGOT | AFSSA |

| | | |
|---|---|---|
| CELER | VLADIMIR | Uni. of Brno |
| CEOLIN | CHIARA | IZS-Ve |
| CETRE-SOSSAH | CATHERINE | CIRAD |
| CHAIGNAT | VALERIE | IVI |
| CHANG | HUIYUN | LVRI |
| CHARREYRE | CATHERINE | MERIAL |
| CHEN | WEIYE | HVRI |
| ÇOKÇALIŞKAN | CAN | ŞAP Enstitüsü |
| CONRATHS | FRANZ | FLI |
| COŞKUNER | AYDIN | ŞAP Enstitüsü |
| COX | SARAH | IAH |
| CRISTALLI | ALESSANDRO | IZS-Ve |
| CULLINANE | ANN | Irish Equine Centre |
| CZUB | MARKUS | Uni. Of Calgary |

**D**

| | | |
|---|---|---|
| DADA | GBENGA AYODELE | Roots and Shoots |
| DALLA POZZA | MANUELA | IZS-Ve |
| DALSGAARD | KRİSTİAN | Biolang |
| DASTJERDI | AKBAR | VLA |
| DAVIS | OLIVER | Fort Dodge |
| DE BATTISTI | CRISTIAN | IZS-Ve |
| DE BOER | MATTHIJN | CVI |
| DE DEKEN | GILL | ITM Antwerp |
| DE KOEIJER | ALINE | CVI |
| DE LEEUW | IISE | VAR |
| DE VOS | CLAZIEN | CVI |
| DEIKE | STEFANIE | HVS |
| DEKKER | ALDO | CVI |
| DELAVERGNE | MORGANE | IFAH-EUROPE |
| DELBECQUE | JACQUES | INGENASA |
| DELILOĞLU GURHAN | S.İSMET | Ege Uni. |
| DEREK | HEALY | VLA |
| DIRBAKOVA | ZUZANA | Veterinary |
| DIXON | LINDA | IAH |
| DOMINGO | MARIANO | CReSA |
| DONALDSON | ALEXANDER | University of London |
| DORTMANS | JOS | CVI |
| DOTTORI | MICHELE | IZSLER |
| DREXLER | CHRISTA | Intervet |
| DUNDON | WILLIAM | IZS-Ve |
| DUPUIS | LAURENT | SEPPIC |

**E**

| | | |
|---|---|---|
| EBLE | PHAEDRA | CVI |
| ELBERS | ARMIN | CVI |
| ENGVALL | ANDERS | SVA |
| ENOE | CLAES | VET-DTU |
| ERGONUL | ONDER | Marmara Uni. |
| ESCHBAUMER | MICHAEL | FLI |

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|---|---|---|
| ESTELA VIOLETA | BLAJ | Pharnachem |
| EVERETT | HELEN | VLA |

**F**

| | | |
|---|---|---|
| FACHINGER | VICKY | Boehringer Ingelheim |
| FAGULHA | TERESA | LNIV |
| FELIZIANI | FRANCESCO | IZSUM |
| FERNANDEZ-PINERO | JOVITA | CISA-INIA |
| FERREIRA | HELENA | CVI |
| FISHBOURNE | EMMA | IAH |
| FOOKS | ANTHONY | VLA |
| FRÖHLİCH | ANDREAS | FLI |

**G**

| | | |
|---|---|---|
| GALE | PAUL | VLA |
| GALL | ASTRID | FLI |
| GALLARDO | CARMINA | CISA-INIA |
| GALLEAU | STEPHANIE | MERIAL |
| GARGILI | AYSEN | Istanbul University |
| GAY | CYRIL | USDA |
| GEARY | STEVEN | Uni. of Connecticut |
| GETHMAN | JÖRN | FLI |
| GIL | PATRICIA | CIRAD |
| GIOVANNI LORIS | ALBORALI | IZSLER |
| GONZALES ROJAS | JOSE L | CVI |
| GRADINARU | DRAGOS | IDEXX |
| GRANSDEN | DORA | QIAGEN |
| GREISER-WILKE | IRENE | HVS |
| GRILLET | COLETTE | CIRAD |
| GRIOT | CHRISTIAN R. | IVI |
| GROSCHUP | MARTIN H. | FLI |
| GRUBMAN | MARVIN | USDA |
| GUIS | HELENE | RAD UMR |
| GULACTI | IREM | Pendik VCRE |
| GULYAZ | VELI | Veterinary |
| GURRALA | RAJESH | VLA |

**H**

| | | |
|---|---|---|
| HAENEN | OLGA | CVI |
| HAGENAARS | THOMAS | CVI |
| HAMERS | CLAUDE | MERIAL |
| HAMMOUMI | SALIHA | CIRAD |
| HAXTON | BEN | VLA |
| HEEGARD | PETER | VET-DTU |
| HENDRICKX | GUY | AVIA-GIS |
| HERR | CECİLE | VAR |
| HOFFMAN | BERND | FLI |
| HULST | MARCEL | CVI |

## I

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|---|---|---|
| IONESCU | AURELIA | IDAH |
| ISABEL MINGUEZ | TUDELA | EC - SDME |

## J

| | | |
|---|---|---|
| JABBAR | AHMED | FZ Borstel |
| JACKOVA | ANNA | Uni. of vet. medicine |
| JALALI | AFSANEH | Svanova Biotech AB |
| JANSSON | EVA | SVA |
| JAROSOVA | VERONIKA | Uni. of Brno |
| JESTIN | VERONIQUE | AFSSA |
| JONG DE | MART | Wageningen UR |
| JONSTRUP | SOREN PETER | VET-DTU |

## K

| | | |
|---|---|---|
| KAARTINEN | LIISA | EVIRA |
| KALTHOFF | DONATA | FLI |
| KARAPINAR | ZEYNEP | Ankara Uni. |
| KEIL | GUENTER | FLI |
| KEITH | SUMPTION | FAO |
| KERKHOFS | PIERRE | VAR |
| KNOWLES | NICHOLAS | IAH |
| KOCH | GUUS | CVI |
| KOENEN | FRANK | VAR |
| KOLBASOV | DENIS | Veterinary |
| KOOI | BART | CVI |
| KOWALCZYK | ANDRZEJ | NVRI |
| KUMAR | MANESH | Fort Dodge |
| KUNTZ-SIMON | GAELLE | AFSSA |
| KUZMAK | JACEK | NVRI |
| KWIATEK | OLIVIER | CIRAD |

## L

| | | |
|---|---|---|
| LARSEN | LARS ERIK | VET-DTU |
| LE POTIER-BLOT | MARIE-FREDERIQUE | LERAPP |
| LEBLANC | NEIL | SVA |
| LECOQ | ANNA | ID VET |
| LELLI | DAVIDE | IZSLER |
| LENS | BART | DiVa |
| LESCEU | STEPHANIE | ID VET |
| LETELLIER | CARINE | VAR |
| LIBEAU | GENEVIEVE | CIRAD |
| LIHONG | LIU | SVA |
| LIPEJ | ZORAN | Croatian vet. institute |
| LIPOWSKI | ANDRZEJ | NVRI |
| LOBOVA | DANA | Uni. of Brno |
| LORENZO | GEMA | CISA-INIA |

**M**

| | | |
|---|---|---|
| MA | JUNWU | LVRI |
| MADSEN | MOGENS | Dianova |
| MARAL | KOREL | Fort Dodge |
| MARKOWSKA-DANIEL | IWONA | NVRI |
| MARTINEZ | DOMINIQUE | CIRAD |
| MARTINEZ | MARTA | CISA-INIA |
| MATERNIAK | MAGDALENA | NVRI |
| MATHEY | ALEXANDER | FLI |
| MATRAS | MAREK | NVRI |
| MEROC | ESTELLE | VAR |
| MERZA | MALIK | Svanova Biotech AB |
| METREVELI | GIORGI | SVA |
| MINTIENS | KOEN | Vose Consulting |
| MISHCENKO | VLADIMIR | ARRIAH |
| MOENNIG | VOLKER | HVS |
| MOJZIS | MIROSLAV | Veterinary |
| MOLLER | KRISTIAN | VET-DTU |
| MOORMAN | ROB | CVI |
| MORENO MARTIN | ANA MARIA | IZSLER |
| MORROW | ALEX | DEFRA |
| MOURAO | ANA | LNIV |

**N**

| | | |
|---|---|---|
| NICZYPORUK | JOWITA | NVRI |
| NIETO | JOSE | CISA-INIA |
| NOVOSEL | DINKO | Croatian vet. institute |
| NOWOTNY | NORBERT | Vet. Uni. Wien |

**O**

| | | |
|---|---|---|
| O'BRIEN | DECLAN | IFAH-EUROPE |
| OGUZOĞLU | TUBA CİGDEM | Ankara Uni. |
| OLESEN | NIELS JORGEN | VET-DTU |
| OOSTERGA-LAND | JITTY | CVI |
| ORTEGO | JAVIER | CISA-INIA |
| ORVEILLON | FRANCOIS-XAVIER | Boehringer Ingelheim |
| OUDE OPHUIS | PETER | DiVa |
| OZAN | EMRE | Samsun VKAE |
| ÖZDURAL | NİLGÜN | ŞAP Enstitüsü |
| OZKUL | AYKUT | Ankara Uni. |
| ÖZYÖRÜK | FUAT | ŞAP Enstitüsü |

**P**

| | | |
|---|---|---|
| PAISLEY | LARRY | VET-DTU |
| PARIDA | SATYA | IAH |
| PARLAK | ÜNAL | ŞAP Enstitüsü |
| PAWESKA | JANUSZ | NICD |
| PEJSAK | ZYGMUNT | NVRI |
| PERRIN | AURELIE | AFSSA |

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| PHIPPS | PAUL | VLA |
| PODGOSKA | KATARZYNA | NVRI |
| POURQUIER | PHILIPPE | ID VET |
| POWELL | LAURA | VLA |

## Q

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| QIU | CHANGGING | LVRI |

## R

| | | |
|---|---|---|
| RAMOS | FERNANDA | LNIV |
| RANALDI | GAELLE | LSI |
| RANZ | ANA | INGENASA |
| RASMUSSEN | LASSE DAM | VET-DTU |
| RASMUSSEN | TANYA | VET-DTU |
| RASMUSSEN | THOMAS BRUUN | VET-DTU |
| REICHERT | MICHAEL | NVRI |
| REID | SCOTT | IAH |
| REVIRIEGO GORDEJO | FRANCISCO | EC - DG SANCO |
| RIJN | PIET A. | CVI |
| RIKULA | ULLA | EVIRA |
| RIZAC | VLADIMIR VASILE | Botosani |
| RODRIGUEZ | FERNANDO | CReSA |
| RODRIGUEZ | LUIS | USDA |
| ROEURMUND | HERMAN | CVI |
| ROLA | MARZENA | NVRI |

## S

| | | |
|---|---|---|
| SALT | JEREMY | Pfizer animal health |
| SAREYYÜPOĞLU | BEYHAN | ŞAP Enstitüsü |
| SAWYER | JASON | VLA |
| SCHENNING | ALEKSANDRA | Svanova Biotech AB |
| SCHROEDER | SABINE | HVS |
| SCICLUNA | MARIA TERESA | IZSLT |
| SEITZER | ULRIKE | FZ Borstel |
| SELLAL | ERIC | LSI |
| SELLERS | SCOTT | DEFRA |
| SERVAN DE ALMEDIA | RENATA | CIRAD |
| SHENDRIK | ALENA | National institude |
| SMIETANKA | KRZYSZTOF | NVRI |
| SMITS | MARI | CVI |
| SMRECZAK | MARCIN | NVRI |
| SOTELO | ELENA | CISA-INIA |
| SOZZI | ENRICA | IZSLER |
| STADEJEK | TOMASZ | NVRI |
| STAHL | KARL | SVA |
| STARICK | ELKE | FLI |
| STAUBACH | CHRISTOPH | FLI |
| STENFELDT | ANNA CAROLINA | VET-DTU |
| STIRLING | JULIE | IAH |

| | | |
|---|---|---|
| STOCK | MANFRED | PIK Potsdam |
| STOCKHOFE | NORBERT | CVI |
| SULTAN | GULCE IZ | CReSA |
| SUSZYNSKI | MARCIN | Vetco.org |

## T

| | | |
|---|---|---|
| TAMM | STAFFAN | SVA |
| TAMPAKIS | KONSTANTINOS | QIAGEN |
| THIAUCOURT | FRANCOIS | CIRAD |
| THUER | BARBARA | IVI |
| TISSERAND | FLAVIA | LSI |
| TOPLU | NIHAT | Uni. of Adnan |

## U

| | | |
|---|---|---|
| UNSAL BACA | AYSEL | Pendik VCRE |
| UTTENTHAL | ASE | VET-DTU |

## V

| | | |
|---|---|---|
| VAHLENKAMP | THOMAS | FLI |
| VAN BORN | STEVEN | VAR |
| VAN DEN BERG | THIERRY | VAR |
| VAN DER LAAG | PETRA | CVI |
| VAN DER POEL | WIM H. M. | CVI |
| VAN DER STEDE | YVES | VAR |
| VAN WUYCKHUISE | LINDA | Animal Health Service |
| VANBINST | TINE | VAR |
| VANDEMEULEBROUCKE | ELISE | VAR |
| VANDENBUSSCHE | FRANK | VAR |
| VANNIER | PHILIPPE | AFSSA |
| VARGA | GABRIEL | Fort Dodge |
| VATANSEVER | ZATI | Kafkas Universitesi |
| VEDDER-ROOTJES | MARGRIET | Wageningen UR |
| VERONESI | EVA | IAH |
| VOIGHT | ULLRIKE | Fort Dodge |
| VOLPE | CARMELO | Smiths detection |
| VONK NOORDEGRAAF | COR | Intervet |
| VOSLOO | WILNA | CSIRO |
| VRANCKEN | ROBERT | VAR |

## W

| | | |
|---|---|---|
| WAELEN | SUSANNE | LNV |
| WAHLSTROM | HELEN | SVA |
| WAKELEY | PHILIP | VLA |
| WEESENDORP | EEFKE | CVI |
| WELBY | SARAH | VAR |
| WESTERGAARD | JORGEN | ADC-Consult |
| WIERINGA-JELSMA | TINKA | CVI |
| WIJASZKA | TADEUSZ | NVRI |
| WILKING | HENDRIK | FLI |
| WILLEBERG | PREBEN | Uni. of California |
| WILSON | ANTHONY | IAH |

| | | |
|---|---|---|
| WITTING | MARCELLA | USDA |
| WOOD | JOHN | Qefii Hellas |
| WOZNIAKOWSKI | GRZEGORZ | NVRI |

**Y**

| | | |
|---|---|---|
| YIN | HONG | LVRI |

**Z**

| | | |
|---|---|---|
| ZAKHAROV | VALERY | ARRIAH |
| ZHANG | YUN | HVRI |
| ZHANG | ZHIDONG | IAH |
| ZHIAGO | BU | HVRI |
| ZIENTARA | STEPHAN | AFSSA |
| ZOHARI | SIAMAK | SVA |

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## W



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## Z



**Contact:**

Web: www.epizone-eu.net
E-mail: epizone.cvi@wur.nl
Phone: +31 320 23 88 83

**Mission of EPIZONE**

EPIZONE is an EU funded Network of Excellence for Epizootic Disease Diagnosis and Control to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks of Foot-and-mouth disease, Classical swine fever, Avian influenza, and other relevant epizootic diseases like Bluetongue and African swine fever, through increased excellence by collaboration.