

EPIZ

Abstracts

5th Annual Meeting EPIZONE "Science on alert"

Satellite Symposium "Bluetongue and other vector borne diseases"

11-14 April 2011, Arnhem, The Netherlands

Hosted by CVI

Network of Excellence for Epizootic Disease Diagnosis and Control



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5th Annual Meeting EPIZONE 'Science on Alert' 11 April – 14 April 2011 Wageningen The Netherlands

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The complete abstract book and all posters can be found on the usb-stick that will be handed out at the registration desk.

EPIZ

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Welcome





Welcome

Dear participants,

We welcome you all to the 5th Annual Meeting of our EU funded Network of Excellence, EPIZONE

"Science on alert"

The slogan "Science on alert" relates to different aspects of preventing and combating animal diseases, in relation with food safety and climate change. Quick, adequate and socio-acceptable actions are needed to control new outbreaks of animal diseases in Europe. Scientists need to be alert to signal all changes in the environmental situation, and respond with quick and specific diagnosis of suspicions, and effective control measures to reduce losses as much as possible.

A main objective of EPIZONE is to interact with fellow scientists. During this meeting you will meet scientists from many different fields of research, but all share their interest in animal disease control. This will give you the chance to discuss ideas and thoughts, to learn from each other, to build new relationships and to strengthen the existing ones.

The Welcome session on Tuesday morning will be dedicated to "How veterinary research relates to animal health policies". Keynote speakers from different organizations will discuss the relation between scientists and policy makers. During the complete meeting, special attention will be given to the outcomes of five successful EPIZONE years, besides the oral presentations on the EPIZONE themes related to recent research.

Satellite symposium

"Bluetongue and other vector-borne diseases"

A major part of Wednesday will be reserved for the latest developments on Bluetongue and other vector borne diseases, with a total of 60 oral presentations and posters on all aspects of the diseases like epidemiology, risk assessment, diagnostics and intervention strategies.

Of course, networking is an important part of this meeting. Therefore we have created an inspiring environment for you, to make you feel comfortable and allow you to interact in a pleasant atmosphere. We wish you all a very successful and enjoyable meeting!

Professor Wim van der Poel, Coordinator EPIZONE

The scientific and the organizing committee

Scientific committee:

Chair: Prof. Wim van der Poel (CVI) Dr. Chris Oura (IAH) Dr. Claes Enøe (VET-DTU) Dr. Elisabeth Erlacher-Vindel (OIE) Dr. Emmanuel Albina (CIRAD) Dr. Franz Conraths Dr. Linda Dixon (IAH) Dr. Yves van der Stede (VAR) Prof. Alexander Donaldson (Bio-Vet Solutions Ltd) Prof. Åse Uttenthal (VET-DTU) Prof. Christian Griot (IVI) Prof. Dirk Pfeiffer (Royal Veterinary College) Prof. Markus Czub (University Calgary)

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VERIA and **BLUETONGUE**

2002

of the frst EU

consortium

in charge of

developing a Bluetongue

vaccination strategy

2004

Industrial partner The First inactivated BTV vaccine with serotype 2

2006

The First inactivated BTV serotype 4 vaccine serotype 9 and the first bivalent vaccine

2008 The First inactivated BTV vaccine

2009

BTVPUR AlSap 8 the frst BTV vaccine to obtain a EUMarketirg Authorisation.

2010 3 New EU Marketing Authorisations BTVPUR AlSap 2-4 **BTVPUR AlSap 1** BTVPUR AlSap 1-8

To date, millions of doses of MERIAL's BTVPUR AlSap have been safely and effectively used and have contributed to successful Bluetongue control programs in 14 European countries.

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Same vaccine fcr sheep and cattle Same injectable dose for both Species

Simple subcutaneous injection

Use from 2.5 months of age

21 – 35 days to immunity from final injection

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Keynote Alexei Zaberezhny





KEYNOTE: CURRENT SITUATION AND METHODS OF CONTROL OF AFRICAN SWINE FEVER

ZABEREZHNY, ALEXEI D.

D.I.Ivanovski Institute of Virology, Moscow, Russia

African Swine Fever (ASF) is an infectious viral disease that causes high economic losses due to necessity of depopulation of pigs in affected areas, sanitary measures, trade restrictions etc. The virus (ASFV) is relatively stable in the unprocessed meat products and environment, thus, large territories are at risk due to free movement of people and products. The ASFV does not affect people and animals except wild and domestic pigs. Some ticks can become infected and carry the virus for years. Adaptation of the virus by changing into less virulent form would mean the threat of an endemic situation in the area. The disease is endemic in domestic and wild pigs in most of sub-Saharan Africa and Sardinia/Italy. There is no treatment for ASF, and no vaccine has been developed. In case of infection with less virulent ASFV strains, recovered pigs could spread the virus as long as thy live. By clinical sympthoms, ASF is very similar to Classical Swine Fever. Methods of laboratory diagnostics are well developed and efficient for identification of ASFV and virus-specific antibodies. Experience of eradication of ASF in Spain suggests the importance of serological monitoring of pigs.

In the spring of 2007 the ASF was detected in Caucasus region. Same virus was detected in Georgia, Armenia, Azerbaijan, and Russia. The ASFV circulating in the Caucasus and the Russian Federation is a highly virulent virus. No reduction of virulence was observed since the first outbreak in Georgia. In the year of 2011, the ASF remained in the Caucasus, southern parts of Russia and appeared occasionally as far as St. Petersburg and region, and in the area of N. Novgorod. Domestic pigs play important role in ASFV spread; they transfer the virus to wild boars. The virus circulates in the population of wild boars depending on their density in the area. Occasionally, the disease is spread from wild to domestic pigs. There is no evidence of ticks being involved in the process. Thus, human activity in raising pigs is largely responsible for continuous spread of the disease. Despite vigorous monitoring and sanitary measures, the disease has not been stopped. The control strategy for ASF should consider international (especially Spanish) experience and local situation. The strategy is based on number of important steps including rapid localization of the disease by trained specialists, setting up buffer zones, constant monitoring of swine population and farms, improvement of diagnostic facilities, training of veterinary personnel, development of system of information and international collaboration.





Theme 4 Diagnostics





ORAL: FIVE YEARS OF HIGH THROUGHPUT REAL TIME PCR DIAGNOSTICS FOR BLUETONGUE IN THE NETHERLANDS

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 CVI^1

Key words: Buetongue PCR-diagnostics outbreak

A real time reverse transcription polymerase chain reaction assay (PCR-test) based on segment S10 of bluetongue virus (BTV) was developed. The PCR-test consists of robotized viral RNA isolation from EDTA-blood samples, and an all-in-one method, that includes predenaturation of dsRNA, synthesis of cDNA (RT), amplification (PCR), and real time detection/analysis. Representatives of 24 known BTV serotypes, isolates of different years, and from different geographic origins, were all detected. Viruses of other serogroups of the genus Orbivirus, like serotypes of African horse sickness virus, epizootic hemorrhagic disease viruses, equine encephalosis virus, and Akabane virus were not detected. Experimentally infected animals were PCR-positive from two days post inoculation, which was earlier than development of clinical signs, and detection of antibodies (Backx et al., 2007, 2009). The diagnostic sensitivity and specificity is approximately 100% as determined with samples from experimentally infected cattle, sheep, and goats. The here described PCR-test has played a key role in the first detection of BTV serotype 8 in August 2006 in North-West Europe. In addition, the PCR-assay has been used to detect BTV in pools of Culioides (Meiswinkel et. al., 2007, Dijkstra et al., 2008). The starting outbreak in a completely naïve and BTV-susceptible ruminant population was taken as an opportunity to validate the PCR-test. In this first BTseason, the correlation between ELISA-positivity and PCR-positivity of field samples was estimated >95%. From 2006 to 2011, the PCR-test was used for testing of animals suspected of Bluetongue, for monitoring and screening programs, and for trade purposes. In total over 200,000 ruminant samples were tested, including these of deer, and exotic ruminants, like camelids. In autumn 2008, the incursion of BTV6/net08 (IAH collection nr. BTV-6 NET2008/05) was discovered by this PCR-test. Starting from August 2008, the genetic variability between the PCR-primers of the amplified sequence was used to evolve this first line of laboratory diagnostics for bluetongue to detect new incursions in the present epidemic. For this purpose, amplicons of the PCR-positive samples were sequenced to confirm the currently circulating BTV8/net06. By use of this second line confirmation or identification, the unexpected BTV6/net08 as well as the northwards expanding, and thus expected, BTV1 was detected. In order to update the PCR-assay, nonvirulent and proposed 25th serotype of the BTV serogroup, Toggenburg Orbivirus (Hofmann et al., 2008), was also detected by this PCR-assay. The performance and up scaling to high throughput 96-format systems in routine use of PCRdiagnostics for bluetongue virus over five years of operation was recorded and will be evaluated.

References

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ORAL: PERSISTENT CLASSICAL SWINE FEVER VIRUS INFECTION IN PIGS

UTTENTHAL, ÅSE¹; LOHSE, LOUISE¹; RASMUSSEN, THOMAS BRUUN¹; NIELSEN, JENS¹

VET-DTU¹

Key words: CSFV, Persistent infection, diagnostics, pig, virus

For the pestivirus Bovine viral diarrhea virus (BVDV) persistent infection (PI) in new born calves is well documented. PI calves are recognized as the main route of spreading and maintaining BVDV infections in cattle populations. In pigs, persistent Classical swine fever virus (CSFV) infection is known but the importance and occurrence of PI piglets is unknown and a discussion. One of the most obvious differences between cows and pigs is that is that cows gives birth to 1-2 calves whereas the sow gives birth to 10-20 piglets. In cases of twin calving, both are usually either PI or non-PI. In swine, however, a litter of piglets may contain mummified fetuses, PI piglets, antibody positive piglets and normal litter mates. The diagnostic methods may give the correct answer.

To study persistent CSFV infection 4 sows were infected by intranasal inoculation using CSFV Lithuania 2009 (100 000 TCID50 per sow) between day 50-60 of gestation. The sows showed only minor clinical symptoms including few days of slight fever and inappetance. CSFV nucleic acid was detected in all 4 sows in serum and/or fecal samples between PID 7-15. At farrowing the sows had high titers of antibodies towards CSFV and were virus nucleic acid negative. The 4 sows gave birth to in total 66 piglets. Thirty-six were live piglets and 30 were stillborn or mummified fetuses.

Blood samples were collected from all live piglets on a weekly basis to follow their level of nucleic acids by quantitative real-time PCR and the level of antibodies in serum. The experiment was ended 33-37 days post partum. Some piglets were euthanized earlier due to welfare reasons, however, 42% of the piglets were still alive at until the end of the experiment.

Some of the piglets had very high virus levels in serum indicating high viral loads (down to Ct 16); as previously seen in piglets infected at 1 week of age (Uttenthal et al, 2008, 7th ESVV Pestivirus symposium, Uppsala). Most piglets had high levels of antibodies as well, suggested to represent a combination of maternally derived antibodies and active immunity.

The piglets could roughly be divided into 3 groups:

A. Possible persistent infection (approx 25%) having high virus levels in serum for several weeks. Some of these piglets showed clinical symptoms such as depression, CNS disturbances and discoloration.

B. Transient CSFV infection of very short duration (about 30%): The pigs were born virus and antibody negative, but seroconverted shortly after ingestion of colostrum. Not all piglets were shown to be viremic but as blood was only collected weekly they may have had a few days of un-noticed viremia. These piglets were eventually infected by the PI littermates but protected from clinical disease and prolonged viremia by the high level of passively acquired antibodies.

C. Severely affected piglets that died within the first week after birth (about 45%). These pigs may be infected in utero or just after birth. Their status need to be defined.

The course of infection and the interpretation of diagnostic tools for determination of PI in pigs will be presented and discussed.

Acknowledgement: This study was supported by Directorate for Food, Fisheries and Agri Business in Denmark, grant no 2007-776.



ORAL: IDENTIFICATION AND MOLECULAR ANALYSIS OF BOVINE KOBUVIRUS FROM COWS IN THE NETHERLANDS

<u>BARRY, ALINE¹</u>; HULST, MARCEL¹; VASTENHOUW, STEPHANIE¹; HAKZE VAN DER KONING, RENATE¹; VAN DER POEL, WIM H.M.¹

 CVI^1

Key words: Bovine, diarrhea, RT-PCR, kobuvirus, FMDV

To date bovine kobuvirus has been reported in just a few countries in Europe and Asia. Clinical signs after infection have not been established yet, and viral detection has been described in apparently healthy and diarrheic calves. The virus belongs to the Picornaviridae family, Kobuvirus genus, which also includes human kobuvirus (Aichi virus) and porcine kobuvirus. The genome is composed of a single stranded RNA molecule of 8.3 kb organized in one open reading frame (ORF). This ORF encodes the structural and non-structural proteins, including the viral polymerase gene (RdRp). This study describes for the first time in the Netherlands, the presence of bovine kobuvirus in serum and fecal samples from cattle, and its phylogenetic analyses including other kobuvirus strains. Pooled serum and oral swab samples obtained from cattle showing vesicular lesions in the mouth were screened by microarray using the EPIZONE DNA-chip. The microarray analysis showed a few positive hits. One of these, from an oral swab, revealed kobuvirus. To confirm the result of the microarray assay and identify the kobuvirus strain, a pooled serum sample was tested by RT-PCR with primers targeting the RdRp gene of bovine kobuvirus. We also tested fecal samples from other calves using universal kobuvirus primers. The selected primers were designed based on human, porcine and bovine kobuvirus strains and targeted the viral RdRp gene. RT-PCR products were sequenced in both directions using the same primers employed in the RT-PCR. Molecular and phylogenetic analyses were performed using BioEdit vs 7.0.9 and MEGA 4.1 software, respectively. The microarray detection of the pooled oral swab sample positive for bovine kobuvirus was confirmed by RT-PCR. Two calve fecal samples were also positive and the three amplicons were sequenced. The molecular analysis (130 nt) showed genetic diversity (88.4 to 93%) of bovine kobuvirus strains circulating in the Netherlands. The dendrogram reconstruction including stains detected in Thailand, Japan, Hungary and Belgium placed each sequence of Dutch kobuvirus in a different cluster. In the present study, the detection of bovine kobuvirus in serum, oral swabs, and feces of calves from the Netherlands was demonstrated. The virus was just recently described in no more than two other countries in Europe. Since only a few studies of bovine kobuvirus have been reported, the association between infection and disease is not clear yet. Considering the symptoms of the Aichi virus infection in humans, it is possible that the bovine kobuvirus causes enteric lesions and also leads to development of diarrhea in infected animals. However, other members of Picornaviridae family include several different target replication organs. The samples were all negative for foot and mouth disease virus (FMDV). FMDV and kobuvirus belong to the same family, i.e., they have genetic similarity. Kobuvirus might be present in the mouth and cause oral lesions. These aspects may be considered when diagnosing FMD, since the possibility of cross reaction in diagnostic assays need to be elucidated. The detection of the virus in serum indicates viremia, and the shedding of the virus in feces, indicates at least one route of excretion, but not necessarily the development of symptoms exclusively in the gastrointestinal tract. The genetic variability of the virus, even in a small fragment of a conserved gene, reinforces the need for further molecular studies to characterize circulating strains.



ORAL: THE KOI HERPESVIRUS DISEASE – VIROLOGICAL AND SEROLOGICAL INVESTIGATIONS ON EARLY AND LATE PATHOGENESIS

BERGMANN, SVEN M.¹; OLESON, NIELS JØRGEN²; CASTRIC, JEANETTE³; JANSSON, EVA⁴; ENGELSMA, MARC⁵; HAENEN, OLGA⁵; BOVO, GUISEPPE⁶; MATRAS, MAREK⁷; KEMPTER, JOLANTA⁸

FLI¹; VET-DTU²; AFSSA³; SVA⁴; CVI⁵; IZS-Ve⁶; NVRI⁷; Technical University of Szczecin⁸

Key words: KHVD, virology, serology, pathogenesis

KHV diagnostics is recently limited to virological assays which may fail in terms of a persistent KHV infection with a very weak virus load between five and 10 genomic particles per ml. A virus replication after reactivation in fish is possible mainly when a stress situation occurs, e.g. seasonal temperature and hormone changes, netting for transportation or a rapid food change. KHV is replicated in fish to a reasonable and detectable level between 200 and 1.000 genomic particles measured by quantitative real-time PCR (qPCR) in at least 50 to 70% of the collected carp (Cyprinus carpio) samples. If samples from carp are collected directly after catch, often false-negative results can be observed. To close this diagnostical gap due to inclusion of different virological-molecular biological assays with and without induced stress situations and the host immune response by measuring of production of specific antibodies by serum neutralization assay (SNT) and antibody ELISA, a direct result comparison was carried out. Virus replication, virus re-activation and serological response against KHV with and without clinical signs of a KHVD were determined for early and late pathogenesis. The developed tools allow the detection but also the exclusion of a KHV infection which might be happen without any clinical signs.



ORAL: PRELIMINARY VALIDATION OF A WEST NILE VIRUS-SPECIFIC COMPETITIVE ELISA AND A MAC WEST NILE VIRUS IGM CAPTURE ELISA

<u>POURQUIER, PHILIPPE¹</u>; COMTET, LOÏC¹

 IDVET^1

Key words: West Nile Virus, competitive ELISA, specificity, MAC IgM antibody capture ELISA

The West Nile Virus (WNV) is a single-stranded, positive-sense, enveloped RNA virus that is maintained in nature through a mosquito-bird-mosquito transmission cycle.

A member of the Flaviviridae family, WNV is closely related to other significant human pathogens including yellow fever (YFV), dengue (DENV), tick-borne encephalitis (TBEV), Japanese encephalitis (JEV), Murray Valley encephalitis (MVEV), and St. Louis encephalitis (SLEV) viruses.

WNV is endemic in parts of Africa, Europe, the Middle East, Asia and Australia.

Flavivirus infection may be diagnosed by indirect immunofluorescence staining of infected cells, plaque reduction assay and ELISA.

Since 2006, IDVET has commercialized the ID Screen West Nile Competition ELISA for the detection of anti-WNV antibodies in multiple species, including birds and horses. This kit is well-documented, but studies have shown that it can cross-react with other Flaviviruses. To reduce cross-reactions with other Flaviviruses, IDVET has produced a new competitive ELISA through the use of a different WNV-specific monoclonal antibody.

Specificity was tested on 300 horses from disease-free areas and 50 horses from areas where the Usutu virus is endemic: all sera gave negative results. (Some of the Usutu-positive sera gave false-positive results on the first competitive ELISA.)

Sensitivity was evaluated through the analysis of 20 positive horse sera from the South of France, where WNV outbreaks were observed in 2003 (Camargue) and 2006 (Perpignan): all sera gave positive results.

Serial dilutions of 3 positive sera were tested on both the old and new ELISAs. Results indicate that these tests have equivalent analytical sensitivity.

With equivalent analytical sensitivity and sensitivity for West Nile virus antibodies, the new test shows significantly less cross-reactions with other Flaviviruses.

In addition, further to numerous outbreaks of WNV in Europe in 2010, scientists require tools for the identification of recent infections.

IDVET has therefore developed a MAC (IgM Antibody Capture) ELISA for the detection of WNVspecific IgM antibodies, which appear in the early stages of infection. (The competitive ELISA will detect both IgM and IgG antibodies, but cannot differentiate between the two).

Specificity of the MAC ELISA was evaluated through the analysis of:

- 300 sera from horses from disease-free areas,

- 20 WNV IgG-positive horse sera from the South of France where WNV outbreaks were observed in 2003 (Camargue) and 2006 (Perpignan). (The sera were collected in 2007.)

All sera gave negative results.

Sensitivity was tested through the analysis of 12 sera from recent infections. All sera gave positive results.

The WNV-specific competitive ELISA together with the IgM capture ELISA are valuable tools for WNV disease surveillance. IDVET will extend the validation of these tests to bird samples, and is interested in collaborating with research groups.



ORAL: VALIDATION OF H9 REAL TIME RT PCRS AND HA PHYLOGENETIC STUDIES OF DIVERSE H9 AVIAN INFLUENZA VIRUSES

HANNA, AMANDA¹; GOVIL, JYOTIKA¹; KRILL, DAVIDA¹; MAHMOOD, SAHAR¹; SHELL, WENDY¹; MANVELL, RUTH J.¹; BANKS, JILL¹; BROWN, IAN H.¹; SLOMKA, MAREK¹

 VLA^1

Key words: Avian influenza, H9, RRT PCR, phylogenetics

Avian influenza viruses (AIVs) of the H9 haemagglutinin (HA) subtype are prevalent in many Asian, Middle Eastern and European countries. In contrast to H5 and H7 subtypes of AIV, these H9 viruses are low pathogenic (LP) and non-notifiable, yet are endemic in many Asian and Middle Eastern countries since the 1990s. Due to the less obvious clinical signs caused by H9, it has rapidly spread, causing detrimental effects on the poultry industry and transmission to humans. In addition to the immediate concern of highly pathogenic H5 and H7 subtypes, H9 AIVs also play a role in spreading disease, causing serious impact on the poultry industry and human health.

Consequently, H9 AIV represents a current veterinary problem and there is a clear need for an accurate and sensitive test to enable rapid detection. Two H9 real time RT PCRs (RRT PCRs) have been developed, described by Monne et al (2008) and Shabat et al (2010), targeting the HA2 and HA1 region of the H9 HA gene respectively. The validation of these tests was conducted with a limited number of specimens; therefore, we evaluated these RRT PCRs using a larger and more diverse range of AIV specimens available to us from the Avian Virology Repository at VLA. Presently, the public database is dominated by H9 HA sequences from Asia (particularly China), but there is limited data available from European H9s. In this study we performed HA nucleotide sequencing on 31 H9 isolates.

Overall, 87 samples (61 egg grown and 26 clinical specimens) of H9 subtype were tested using the RRT PCRs described by Monne et al (2008) and Shabat et al (2010). These were of diverse geographical origin including; Asia (n=28, including 25 from the Indian subcontinent), Middle East (n=36), North Africa (n=2) and Europe (n=21). The H9 RRT PCR described by Monne et al (2008) gave comparable Ct values to the M gene RRT PCR (Spackman et al 2002) with all H9 isolates tested. With the H9 RRT PCR described by Shabat et al (2010), only isolates from the Middle East, North Africa and Indian subcontinent were detected. In order to assess the specificity of these RRT PCRs, a representative of all avian influenza HA subtypes, H1-H16, (n=25) were tested along with negative clinical specimens (n=88). All samples were shown to be negative indicating the exclusive specificity of these two RRT PCRs for H9 detection. 31 H9 isolates were amplified by conventional RT PCR and sequenced. These isolates originated from Asia (n=1), Indian subcontinent (n=11), North Africa/Middle East (n=11) and Europe (n=8) and were analysed phylogenetically.

This study has validated two published H9 RRT PCRs using a diverse range of H9 isolates. It is noted that the H9 RRT PCR described by Monne et al (2008) is suitable for detecting a wider range of H9 isolates than described by Shabat et al (2010). The phylogenetic tree generated illustrates clear geographical divergence among isolates from Europe, North Africa/Middle East, China and the Indian subcontinent. The H9 HA gene sequences generated in this study will contribute to the public database, for which European sequences in particular are limited.

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Theme 6 Surveillance and epidemiology





ORAL: THE BELGIAN MOSS: A MONITORING AND SURVEILLANCE SYSTEM FOR THE EARLY DETECTION AND IDENTIFICATION OF (RE-)EMERGING ANIMAL DISEASES

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Key words: Emerging diseases, MoSS, Early detection, Cluster analysis

To ease the early detection of (re-)emerging animal diseases, the Belgian Sanitary Authorities agreed that a system is needed to reduce the delay between the occurrence of first clinical signs of emerging diseases and the identification of the causative agent, to restrict consequences related to outbreaks. Also, when looking at the identification process of Bluetongue in Belgium (2006), a more structured communication between veterinarians - confronted with an emerging disease - and (veterinary) experts needed to be organised. This understanding led to the development of 'MoSS' (Monitoring and Surveillance System); an online epidemiological information system – http://www.moss.be – to centralize and analyse data on clinically suspicious cases. The MoSS aims at i) facilitation of early detection and identification of emerging diseases and ii) development of a strong epidemiovigilance network of veterinarians and experts.

The MoSS is based on active reporting of atypical syndromes by veterinarians, defined as: i) unknown, emerging syndromes, ii) known diseases with an unusual clinical expression and/or non-responding to the usual treatment and iii) rare/re-emerging diseases. An on-line enquiry form is accessible to record information about date of onset of the case, its location, clinical signs and epidemiology. Similar records are grouped in the MoSS using a hierarchical ascending clustering process, followed by an alert signal after the onset of a new cluster of \geq 3 cases. This alarm leads to the selection and invitation of the 'best-fit' expert with regard to the content of the cluster in question, after which communication between the veterinarians and expert(s) is organised on one forum page per identified cluster, connecting all levels of expertise to facilitate the diagnostic approach.

Although the MoSS-website is in the final stage of construction, several records have been made yet in the MoSS. For instance, recorded cases of Bovine Neonatal Pancytopoenia (BNP), a currently emerging syndrome in calves, have been analysed by the system which resulted in a cluster of 78 BNP records, made by 5 observers. This example shows that the BNP cases are recognized as a cluster, irrespective of personal variation between the records. Also, when the BNP cluster was created, none of the remaining records in the database concerned BNP, which illustrates the system's potential to discriminate related cases from the database's surrounding "noise". However, as the clustering method is fully adjustable, changes in parameter settings will lead to alternative clusters. Hence, validation is needed to find the most optimum combination of parameter settings to detect potential emerging syndromes with maximum timeliness and sensitivity.

Based on clinical observations, MoSS relies greatly on the experience and alertness of veterinarians, as well as their goodwill to report unusual findings. Even when the success of the MoSS is proven by experience, it remains a challenge to keep veterinarians motivated to continue submitting data after the newness of the system diminishes. These aspects could result in underreporting, which is not uncommon in passive reporting strategies.

The MoSS is based on the centralization and analysis of information provided by veterinarians and will be a critical tool aiming at shortening the detection time of (re-)emerging diseases. It is a first significant step in the preparedness for detection of the 'unexpected', as well as monitoring of epidemic diseases.



ORAL: OPTIMAL SURVEILLANCE DESIGN FOR LOW PATHOGENICITY AVIAN INFLUENZA IN MEAT TURKEYS

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Key words: Low pathogenicity avian influenza, surveillance, turkeys

Highly pathogenic avian influenza (HPAI) causes massive epidemics in domestic poultry, leading to huge economic losses and threatening human health. Therefore, surveillance and control of avian influenza (AI) have primarily focused on the detection and eradication of HPAI from poultry populations. However, because HPAI viruses may emerge by mutation from low pathogenicity AI (LPAI) viruses of the H5 and H7 subtypes circulating in poultry, monitoring LPAI viruses is important as well. Since the disease dynamics and the clinical picture of LPAI are different from HPAI, surveillance should be optimized specifically for LPAI.

Four LPAI epidemics in turkey flocks in Italy (2000-2005) have provided a wealth of data, with test results of more than 5200 sampling events in 2463 turkey production rounds. We have analyzed a subset of these data to build and parameterize a transmission model of LPAI in turkeys, and to estimate the rate of passive detection of outbreaks in turkey flocks. In the present study we have used this model to evaluate some surveillance scenarios, based on the surveillance scheme in place just before the Italian epidemics. The outcomes of surveillance scenarios were evaluated by comparing the herd reproduction number (Rh, i.e. the average number of outbreaks caused by one infectious herd) of an infected herd at the time of detection, assuming that the herd will be culled or effectively quarantined after that. Because the model describes LPAI transmission in the absence of other control measures such as vaccination or contact tracing, the results will reflect the worst case scenario.

In our baseline model, we assumed a combination of both active and passive surveillance applied to a flock of 10,000 male turkeys in absence of any control measure. Sampling for active surveillance in the baseline model was assumed to start at day 60 of the production cycle and be performed on a monthly basis. At each sampling we assumed that 10 birds were tested by means of both serological and virological assays and one positive result was enough to declare the herd as infected.

The outcome of the baseline model indicated that 82% of the infected herds is detected, on average about one month after the virus introduction, and mainly by active surveillance (74% of detected outbreaks). It also indicated that to prevent an epidemic (i.e. Rh < 1), stopping the between-flock spread by control measures should be achieved immediately after detection (i.e. within 2 days after sampling).

Simulation of alternative surveillance strategies suggests that increasing the sampling frequency every 15 days) or the number of samples (30 birds), allows for two or three more days to stop the between-flock transmission. A little improvement can be obtained also starting the first sampling at day 30 instead of day 60. Conversely, decreasing the sampling frequency (every 60 days), performing only passive surveillance, or applying only serological tests, made it impossible to timely detect the infection to avoid the spread of the virus to other herds (Rh > 1).

The main conclusion from this analysis is that the sampling scheme that was in place in Italy before the first epidemic (monthly sampling 10 birds as from day 60 of the production cycle) is effective if direct action is taken to prevent transmission between flocks. Intensifying the scheme does not greatly improve performance, but the easiest improvement would be to start sampling earlier in the production cycle.



ORAL: PIGS INFECTED WITH PANDEMIC INFLUENZA VIRUS A/H1N1 LACK CROSS REACTIVE HI ANTIBODIES AGAINST EURASIAN SWINE INFLUENZA VIRUSES OF SUBTYPE H1N1

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 FLI^1

Key words: pandemic influenza virus A/H1N1, pigs, hemagglutination inhibitiontest, cross reactivity

Experimental studies have shown that naive pigs are susceptible to pandemic influenza virus A/H1N1 and that the virus readily transmits between them (Lange et al., 2009; Brookes et al., 2010). Whether prior infection of pigs with endemic European swine influenza viruses (SIVs) may offer cross-protection against pandemic influenza virus A/H1N1 infection remained to be elucidated. Kyriakis et al. (2010) investigated the occurrence of serological cross-reactivity with pandemic influenza A/H1N1 after infection of pigs with European SIVs. Hemagglutination inhibiting (HI) antibodies were undetectable after a single infection with European SIVs but they were found in all dually, consecutively European SIV-infected pigs. These data suggest that pigs with infection-induced immunity to different European SIV subtypes may be at least partially immune against pandemic influenza A/H1N1. Broad serological cross-reactivity with pandemic influenza A/H1N1 in European pigs in the field further supports this notion (Dürrwald et al., 2010). In contrast to the previous studies we investigated the pandemic influenza virus A/H1N1 infection-induced cross-reactive immunity in pigs to 10 different endemic European SIVs of subtype H1N1 collected between 1979 and 2010. The results reveal that pigs infected with the pandemic influenza virus A/H1N1 do not develop cross-reactive HI antibodies to any of the Eurasian SIVs even when tested with hyper-immune sera, obtained from pigs reimmunised twice. Although these sera need to be further investigated for cross-reactive VN and NI antibodies, the results so far suggest that pigs infected with pandemic influenza A/H1N1 are not protected against infection with endemic European SIVs.



ORAL: EPIZONE: SPATIAL TRANSMISSION OF SWINE VESICULAR DISEASE VIRUS IN 2006-2007 EPIDEMIC IN LOMBARDY

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Key words: spatial kernel SVDv

Swine Vesicular Disease (SVD) is a contagious disease of pigs sustained by an Enterovirus of the Picornaviridae family. At the end of 2006 a recrudescence of SVD was recorded in Italy and the disease spread widely in the Italian northern Regions, in particular in Lombardy, a region with densely populated pig areas. The SVD outbreaks reported in Lombardy can be grouped in two epidemic periods: the first one lasted from November 2006 to February 2007, the second from May 2007 to October 2007. The purpose of this study was to quantify the between-farm spatial transmission via a spatial kernel in each period of the epidemic. For each outbreak the date of virus introduction was established according to laboratory results and data gathered during outbreak investigations. In fact SVDV in Italy was often subclinical and in the 2006-2007 SVDV epidemic in Lombardy clinical signs have been seldom observed. Often outbreaks were detected just by laboratory testing upon surveillance. Due to the behavior of this disease, we considered as the infectious window of each outbreak the interval between the estimated date of infection and the date of cleaning and disinfection of the premises. Moreover, since the day of infection was not exactly known, it was extended to the corresponding week of the epidemic, and as a consequence the analysis was done with time steps of a week. Farms were assumed to be infected in that week and to become infectious immediately after. Thus it was possible to distinguish between a) the susceptible farms, b) infected farms but still not able to infect other farms, c) infectious farms and d) the removed ones, for each week of the epidemic. A dataset was then prepared containing the distances from each infectious farm to each susceptible farm exposed in that same week and also the distances from each infected farm to the infectious farms in that same week per week for both the epidemic periods. The kernel was estimated as $p=\lambda 0/(1+((d/d0)^{alpha}))$. The spatial kernel parameters point estimates and the respective confidence intervals were estimated by maximum likelihood. For the 2006-2007 epidemic period the estimate of the transmission rate for week was 0.02 (95%CI: 0.005-0.22), the power was 1.84 (95%CI: 1.47-2.21) while the estimate for the distance parameter was 520 (95% CI 80-1560). For the 2007 epidemic period the estimate of the transmission rate for week was 0.03 (95%CI 0.009-0.3), the power was 2.4 (95%CI 1.85-3.1) while the estimate for the distance parameter was 1050 (95% CI 210-2240).

This analysis has brought to a better insight in spatial spreading of SVDV in Lombardy in 2006-2007. The short distance transmission seems to be important for SVDV in both the two epidemic periods. However, the transmissions in 2007 is shifted to shorter distance with respect to the first epidemic period outbreak and neighborhood transmission is thus more important for the second epidemic period. This more local transmission in 2007 could be explained by an earlier detection of outbreaks that led to more effective restriction of movements, by improved biosecurity measures and at last preemptive depopulation while in 2006 the disease spread undetected for at least one month and long distance transmission by means of animal transport could occur. According to our results the adoption of strict control measures appeared to restrict the outbreak to a more local area.

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ORAL: EPIZONE: TRANSMISSION OF FMDV WITHIN AND BETWEEN SPECIES: QUANTIFICATION AND COMPARISONS

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Wageningen University¹; CVI²; IAH³

Key words: FMDV, transmission, experimental epidemiology

Data on experimental transmission of FMDV between animals have been collected for a long time. In the previous century several countries have built special facilities to study transmission and other aspects of FMDV infection. The latter experiments sometimes also allowed observation of transmission events. More recently techniques have been developed for statistical inference on experimental data on transmission in small groups of animals. Therefore, we have undertaken to collect and statistically analyse existing data on transmission of FMDV. We focussed first of all the transmission of the virus in unvaccinated animals and on both within-species and between-species transmission. As far as this was in the same datasets we also looked at the consequences of vaccination on transmission. The species that were studied were cattle, pigs and sheep as these are the major livestock species affected by FMDV.

The results of our data collection and analyses can be summarised in a 3x3 matrix where either transmission rate parameters or transmission ratios are the matrix elements. Not all different combinations are studied equally well, but by assuming separability between infectivity and susceptibility of each of the species we could for example infer that transmission from cattle to pigs has a low transmission rate, whereas it was observed that transmission from pigs to cattle has a high transmission rate.

The data apply to transmission between individual animals but for the control of FMDV quantitative data for the transmission rates between farms with the same or different species is even more important. We will therefore discuss the possibility of extrapolating from the between-individual rates to between-farm rates. In addition we will discuss the data gaps and how we could attempt to fill these.

Acknowledgement: We thank all members of EPIZONE WP6.3 for their contribution.



ORAL: A METAPOPULATION MODEL TO SIMULATE WEST NILE VIRUS CIRCULATION IN SOUTHERN EUROPE AND THE MEDITERRANEAN BASIN

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Key words: West Nile virus, model, Europe, Africa

In Europe, recent studies suggest that a recurrent circulation of West Nile Virus (WNV) could exist in some areas. Whether this circulation is permanent due to overwintering mechanisms or not remains unknown. The current conception of WNV epidemiology combines an enzootic WNV circulation in tropical Africa with seasonal introductions of the virus in Europe by migratory birds. The objectives of this work were to (i) model this conception of WNV global circulation; (ii) evaluate whether the model could reproduce data and patterns observed in Europe and Africa.

The model is a deterministic discrete time meta-population model with a daily time step. The epidemiological system is represented by a set of host populations that share during their annual life cycle a set of locations where vector live. Incidental hosts (sentinel chickens and horses) also are living in each location and are exposed to infectious bites. Three locations were considered: a wet African area, a dry African area, and a European Mediterranean area. Three resident bird populations live in these areas, as do three vector populations. Two migratory bird populations link the three areas: long distance migrants (wet African area). Population dynamics of both vectors and hosts as well as Infection dynamic parameters were fixed according to literature data. Age-specific bite relative risk and site-specific vector-host ratios were estimated using published seroprevalence data coming from these 3 zones. Two age classes were considered for the variations of the bite relative risk: nestlings, chosen as the reference class, and flying individuals (juveniles and adults). A systematic univariate sensitivity analysis was conducted to study the effects of parameter variations on the estimated values of the juvenile and adult bite relative risk, and of site-specific vector-host ratios.

The model was validated using independent published studies. According to this model, overwintering mechanisms are not needed to reproduce the observed pattern. However, the existence of such mechanisms cannot be ruled out. This model will be used to test the efficiency of several surveillance system (sentinel horses and chickens, mosquito trapping) as well as climatic scenarios.



Keynote James Maclachlan




KEYNOTE: THE HISTORY AND GLOBAL EPIDEMIOLOGY OF BLUETONGUE VIRUS INFECTION

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Bluetongue (BT) is an insect-transmitted virus disease of ruminants caused by BT virus (BTV). The disease was first described in southern Africa after European settlers introduced their finewool breeds of sheep to the region in the 17th century. For much of the 20th century BT was considered a disease of Africa that was being silently spread around the world, putatively by livestock trade and movement. It is now evident that BTV infection is endemic on all continents except Antarctica, coincident with the distribution of competent *Culicoides* insect vectors that occur in relatively distinct global ecosystems that include different *Culicoides* species and different constellations of BTV serotypes. Climate change has been incriminated as the cause of the remarkable recent expansion of the virus' global range, notably in Europe.

Modified live (MLV) BTV vaccines were first developed in South Africa and subsequently in California. The egg propagated MLV vaccines developed in California were highly teratogenic if used in pregnant sheep and caused characteristic brain defects in congenitally infected progeny. Until the recent emergence of BTV serotype 8 (BTV8) in northern Europe, congenital BTV infection only was described only in regions where MLV vaccines are also used; this includes teratogenic defects in calves born to unvaccinated cattle that were potentially exposed to naturally circulating vaccine viruses or reassortants thereof. The European strain of BTV8 is commonly associated with vertical transmission, which is not a feature of many other field strains of the virus, and BTV8 causes teratogenic brain defects that are identical to those caused by the original California MLV BTV vaccines.

Much attention has been paid recently to the epidemiology of BTV infection in Europe, especially in regions devoid of *Culicoides imicola*, which is the traditional African-Asian vector of BTV. However, much remains to be understood regarding the epidemiology of BTV infection in historically endemic regions such as South Africa and California. Infection in these areas is seasonal, and the mechanism of inter-seasonal maintenance (so-called "overwintering") remains uncertain. Because of renewed international interest in the epidemiology of BTV infection, including the potential role of vertical and/or oral transmission of the virus therein, we have undertaken intensive surveillance at commercial dairy farms throughout California. These studies have confirmed oral infection of newborn calves via consumption of virus-contaminated pooled colostrum, however the presence of BTV nucleic acid is transient in these calves and we have confirmed neither persistent infection nor interseasonal maintenance of BTV in cattle. Rather, data obtained to date confirm only that the annual infection of cattle in California is dependent on seasonal transmission by vector *Culcioides sonorensis* insects.





Theme 5 Intervention strategies





ORAL: RECOMBINANT CAPRIPOXVIRUSES EXPRESSING PROTEINS OF RIFT VALLEY FEVER VIRUS: EVALUATION OF THE IMMUNOGENICITY AND PROTECTION OF THESE VACCINE CANDIDATES

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CIRAD¹; Institut Pasteur²

Key words: RVF, MBT/Pas, Capripoxvirus, Vaccine

Rift Valley fever (RVF) is an arthropod-borne disease caused by a virus of the Bunyaviridae family, genus Phlebovirus. RVF virus (RVFV) is transmitted mostly by direct contact or by mosquitoes of the genera Aedes and Culex, although other arthropods may play a role in its spread. In natural ecosystems, RVF affects mainly sheep, cattle, goats, and humans, but other mammals, such as camels and buffaloes, may exhibit the disease. RVF outbreaks represent a threat for humans in endemic areas, where people may be infected by direct contact with animals or possibly raw milk and by mosquitoes. Originally present in Eastern and Southern Africa, RVFV has spread in recent years to Western Africa, Madagascar, and even outside Africa to Saudi Arabia and Yemen. Several types of vaccines such as attenuated and inactivated ones have been already developed to face epizootics. Secondary effects such abortion, teratogenic effect or virulence reversion may occur with these types of vaccines. The development of recombinants vaccines may have an interest. Protective efficacy has been demonstrated with recombinant capripoxviruses vaccines against Peste des Petits Ruminants and Rinderpest. The objective of our study was the development of protective RVF recombinant capripoxvirus vaccine candidates. Thermostability and one single shot injection are the major advantages of these capripoxviruses used as vaccine vectors. MBT/Pas mice exhibiting an extremely high susceptibility to experimental infection with virulent RVF strains were immunized with single RVF recombinant capripoxvirus expressing the NSmGN proteins. Protection after a virulent challenge was achieved and immune responses were evaluated and will be illustrated in this study.



ORAL: HPAIV H5N1 VIRUS ESCAPED FROM NEUTRALISING IMMUNE PRESSURE BY UNUSUAL VARIATION OF THE NEURAMINIDASE ENCODING SEGMENT

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 FLI^1

Key words: HPAIV H5N1, neuraminidase, virulence, immune escape

Partial immunity normally leads to antigenic drift by selectively favouring viral escape mutants that probably already exist in the quasispecies representing the ancestor virus. These escape mutants should comprise mutations that conserve or better increase antigenic changes in the surface glycoproteins. To investigate this phenomenon, highly pathogenic avian influenza virus (HPAIV) of subtype H5N1 was serially passaged 50 times in embryonated-egg culture under the selective pressure of a neutralising, polyclonal chicken-derived antiserum. While a significant escape from neutralization could be observed, it was not possible to generate a virus completely escaping from being neutralized by a high titer polyclonal antiserum. Fulllength genome sequences of the resulting mutant as well as of a control virus passaged without serum pressure were determined by deep sequencing and further analysed. Comparison of the sequence data revealed major changes especially in the neuraminidase encoding segment of the selected virus mutant. Interestingly, the in vitro-generated novel HPAIV H5N1 virus comprised only of about half of the neuraminidase encoding segment with several frame shifts and an open reading frame of only about 50 amino acids. The sequence changes of this new influenza virus - named "H5N1del MaDo" - were associated with an only slightly reduced in vitro growth, but a complete loss of virulence for chicken (intravenous pathogenicity index = 0). Nevertheless, the cleavage site of the hemagglutinin of "H5N1del MaDo" turned out to be unchanged defining this virus still as potentially highly pathogenic. Interestingly, electron microscopy analysis did not show any unusual accumulation of virus particles at the cell surface in comparison to wild type HPAIV H5N1.

Based on our data, we propose that escaping the neutralising antibody pressure is also possible by establishing major sequence deletions/variations within the influenza A virus segment 6. Additionally, the generated escape virus mutant is the first "neuraminidase-negative" influenza virus, which is historically well documented, and generated without any external neuraminidase supplement. Further in vitro and in vivo analysis of this unique influenza virus are performed and will allow better insights into the function and role of the neuraminidase of influenza A viruses and its role for virus replication and pathogenesis.



ORAL: DIFFERENT POPULATIONS OF ANTIGEN PRESENTING CELLS REGULATE THE PROLIFERATION AND CYTOKINE PATTERNS OF INFLUENZA SPECIFIC MEMORY T CELLS

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 SVA^1

Key words: Influenza, Cytokines, APC, Memory, T cells,

When Balb/c mice were infected with 104 TCID50 of H1N1 PR8 influenza viruses they lost a maximum of 15-20% weight that peaked on days 9 to 10 post infection. Then the mice returned to normal weight within four days. Examined lungs showed severe pulmonary inflammation at the peak of disease where the bronchiolar mucosa was damaged and infiltrated with leukocytes, predominantly polymorphs and macrophages. Interestingly, intranasal administration of IgY antibodies against highly pathogenic H5N1 influenza virus protected the mice against infection with the H1N1 PR8 influenza virus strain, demonstrating that IgY to H5N1 can indeed cross protect against infection with H1N1. Three months later both groups of mice were re-infected with a 100 times higher dose of the homologous influenza virus. No reduction of weight was observed in the animals indicating the development of a protective immunological memory to influenza in the untreated as well as the IgY treated mice.

At 36h post virus challenge their lungs and spleens were rich in CD62Lhigh memory CD4+ T cells and CD8+ Т cells producing the high concentrations of IFN-y. In vitro challenge of immune T cells from "memory" mice with different APCs from infected lung showed that CD4+ influenza specific memory T cells co-cultured with CD11c+ dendritic cells and Gr-1+ granulocytes from infected lung were strongly inhibited in their proliferation. The CD4+ memory T cells still produced significant to high concentrations of IL-2 and proinflammatory IFN-y and IL-17 and delivered efficient co-stimulation to influenza sensitized naïve T cells.

Virus specific CD8+ memory T cells reacted differently compared to CD4+ T cells and exhibited a significant T helper cell independent proliferation in co-cultures with influenza infected lung cells and produced high concentrations of IFN- γ .

In conclusion, IgY anti-influenza antibodies can be used to control influenza virus infection without interfering with the development of adaptive immunological memory, and challenge of immune mice with virus rapidly induce memory T cells in lung and spleen that produce high concentrations of pro-inflammatory IFN- γ and IL-17. Furthermore, different populations of influenza presenting cells in the lung regulate the immune response of influenza specific memory T cells differently in vitro.



ORAL: EPIZONE: TESTING DIFFERENT PRIME, BOOST STRATEGIES FOR DELIVERY OF AFRICAN SWINE FEVER VIRUS ANTIGENS

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Key words: African swine fever virus, vaccine development, viral vectors

Currently no vaccine is available for African swine fever virus and this limits the options for disease control. Previous work has shown that pigs inoculated with attenuated virus strains can be protected from challenge with related virulent strains. In addition partial protection has been achieved using combinations of 3 antigens delivered as recombinant proteins or as DNA vaccines. In this Epizone funded internal call we:

1) Tested a different vaccination regime and measured host responses to an attenuated ASFV strain. Responses measured included T cell, antibody and acute phase protein responses. 2) Tested different prime, boost strategies for delivery of 3 ASFV antigens that have previously been shown to induce partial protection in pigs. The different strategies included priming with recombinant DNA, Bacmam, MVA, adenovirus 5 and boosting with the 3 recombinant proteins. In addition MVA priming and adenovirus boosting was tested.

3) Standardised a clinical scoring system.

4) Provided materials for validation of diagnostic tests.



ORAL: BAC DNA OF RECOMBINANT MAREK'S DISEASE VIRUS (MDV) CONFERS PROTECTION AGAINST VERY VIRULENT PLUS STRAIN OF MDV

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 $NVRI^1$

Key words: Marek's disease, MDV deletive strain, BAC DNA vaccine

Introduction

Marek's disease virus (MDV) is an aetiological agent of Marek's disease in chickens and turkeys that still presents a serious threat in poultry production. MDV encodes two important oncogenes. The first one is a basic leucine-zipper protein, Meq and the second is the viral telomerase (vTR). The previous study conducted by other researchers have shown that MDV strains lacking these oncogenes are capable to replicate well in visceral organs and feathers follicles and could be good candidates for the new vaccines against the disease.

Aim of study

The aim of this study was to establish whether vaccination of 1-day old chickens with BAC DNA extracted from $31/07\Delta$ Meq Δ vTR strain is safety for chickens and could provide a protection against very virulent plus (vv+MDV) strains.

Material and methods

The 31/07 strain was originally isolated from the 27-week-old chickens with tumors in visceral organs. Recombinant strain 31/07 Δ Meq Δ vTR, which lacks the meq and vTR oncogenes, was generated by insertion of pDS-pHA plasmid into the US2 region of MDV genome and Rec E/T recombination in E.coli GS1783 cells. BAC DNA was extracted from 109 GS1783 E.coli cells using Plasmid Maxi Kit (Qiagen) and was used for vaccination of chickens. The commercial vaccine contained the strain CVI988/Rispens was used for the comparison.

One hundred and twenty SPF chickens were separated into six groups.

All chickens from the Ist and IIIrd group were vaccinated in the 1st day of life with DNA BAC $31/07\Delta$ Meq Δ vTR (20 µg in PBS buffer), chickens form the IInd and IVth group were vaccinated in the 1st day of life with the commercial vaccine CVI988 (1400 PFU), Vth and VIth were control groups. Chickens from the Ist, IInd and the Vth group were placed in the room with 8-week-old SPF hens after infection with vv+MDV 31/07 strain and with clinical symptoms of Marek`s disease. Control chickens from the VIth group were non-vaccinated and non-infected. The period of experiment was 28 days. In the 3rd, 7th, 14th, 21st, 28th day 4 chickens from the each group were examined for MD lesions. The visceral organs were collected for the further study. Morbidity and mortality was determined for the each group. Vaccinal protective index (PI) was also calculated.

Results

The protection efficacy of two vaccines types BAC DNA $31/07\Delta$ Meq Δ vTR and commercial CVI988/Rispens were compared. The chickens from the Ist, group have shown 10% mortality after challenge and 20% of changes manifested as an enlargement of spleen while chickens from the IInd group have shown 5% mortality and 5% of anatomopathological changes. The PI of these two vaccines was 90 for vaccine BAC DNA $31/07\Delta$ Meq Δ vTR95 and 95 for commercial vaccine CVI988. However in case of experimental BAC vaccine the incidence of MD anatomopathological changes in chickens was 4 times higher than in case of chickens vaccinated with the commercial vaccine. The new BAC vaccine was safety for chickens similarly to commercial vaccine. All chickens from the Vth group died and showed MD specific lesions.

Conclusion

The recombinant $31/07\Delta$ Meq Δ vTR BAC DNA vaccine elicts protection against vv+MDV however the PI was lower and the incidence of anatomopathological changes was higher than in chickens vaccinated with the commercial vaccine.



ORAL: EPIZONE: CHARACTERIZATION OF THE IMMUNOSTIMULATING POTENTIAL OF CALICIVIRUS CHIMERIC VIRUS-LIKE PARTICLES (VLPS) DISPLAYING FOREIGN B AND T-CELL EPITOPES

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CISA-INIA¹; CRESA²

Key words: Virus-like particles VLPs, Calicivirus, RHDV, T-cell and B-cell epitope

Our research group has developed a system for the production of large amounts of rabbit hemorrhagic disease virus (RHDV) VLPs in insect cells (1). We have identified three independent locations within the gene of the capsid protein (VP60), where we can insert foreign sequences spanning at least 42 aminoacids in length without affecting the ability of the resulting chimeric protein to self-assemble into VLPs. Our aim is to explore the feasibility of using RHDV-derived VLPs as antigen-presenting vectors to induce an efficient immune response against foreign B and T-cell epitopes.

We have generated sets of recombinant baculoviruses expressing VP60 constructs harbouring immunogenic epitopes at different insertion sites: i) a well-defined cytotoxic T-cell epitope derived from ovalbumin protein (OVA); ii) a cytotoxic T-cell epitope derived from influenza virus nucleoprotein (NP366–374); iii) peptides containing the main FMDV B-cell antigenic site and a major T-helper epitope from this virus; iv) a neutralizing B-cell epitope derived from feline calicivirus (FCV).

All the chimeric VP60 proteins prepared assembled into VLPs. We then analyzed the ability of the different chimeric VLPs to induce immune responses against the inserted foreign epitopes in different animal systems. Our results demonstrated that in vitro, chimeric VLPs were able to stimulate dendritic cells and they were processed and presented to specific T cells. In vivo, mice immunized with the chimeric VLPs without adjuvant were able to induce specific cellular responses mediated by cytotoxic and memory T cells. More importantly, immunization with chimeric VLPs was able to resolve an infection by a recombinant vaccinia virus expressing OVA protein (2), or to reduce the severity of symptoms induced by a challenge with influenza virus. The chimeric RHDV VLPs were also shown to induce potent neutralizing responses against foreign B-cell epitopes. The constructions harbouring foreign T-cell epitopes at the N-terminus, which is predicted to be buried in the internal face of the VLPs, exhibited a higher degree of immunogenicity for cell-mediated responses. Conversely, the constructions harbouring foreign B-cell epitopes at an exposed loop (between amino acid positions 306 and 307 of VP60) exhibited best results in terms of the induced humoral responses.

In conclusion, RHDV derived-VLPs constitute versatile scaffolds for antigen display, as these VLPs were able to accommodate simultaneous insertions of foreign epitopes at three different insertion sites. Moreover, the immunogenic properties of the chimeric RHDV VLPs suggest the potential suitability of these constructions for new vaccine development against animal and human viral infections.



ORAL: ICONZ: A PUBLIC HEALTH TEMPLATE FOR THE ENDEMIC ZOONOSES?

SCUDAMORE, JIM

University of Liverpool

The uncertainties of the 21st century require a "new culture of collaboration". Public health is moving towards global, systemic and ecologically sound approaches to natural resource management. "Healthy ecosystems" frame human health and wellbeing in the context of an ecosystems approach where healthy people and healthy environments are inextricably linked. Across Africa there is inadequate assessment of the dynamics of human activities and their impact on local ecosystems. This presents a challenge for low resource countries where livestock production for better human heath demands holistic improvements in human, livestock and ecosystem health. The forgotten diseases, many of which are endemic zoonoses impose a significant but underestimated burden on these communities. To address these challenges human, animal, and ecosystem health must be viewed as a cooperative endeavour between health practitioners and environmental scientists in a collaborative and synergistic effort. This theme of using control methods for the forgotten diseases affecting communities that may have additional benefits a driver for promoting intersectoral collaboration underpins ICONZ. ICONZ is a 7.7M FP7 project looking at how this can be achieved for other endemic diseases affecting animals and people in Africa. It involves the collaboration of 21 universities and research institutes working on case studies of zoonotic disease clusters in seven African countries. This is the first large collaborative project of this nature targeting the forgotten zoonotic diseases, and it is hoped that our understanding of diagnostics, burden of disease and intervention options for their control will be much improved as a result of the case studies. It is hoped that the ICONZ case study framework will deliver significant contribution in the institutional context of disease control - showing how control strategies can fit into an enabling multi-sectoral funding and organisational structure. ICONZ is pulling together Animal Health + Development + Human health people to achieve the necessary drivers for change.





Theme 7 Risk assessment





ORAL: EPIZONE: INTERACTIVE SESSION ON CURRENT EPIDEMIC THREATS

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Key words: epidemic threats, EPIZONE, prevention, control, expert opinion

The EPIZONE network is a European Union (EU) funded Network of Excellence, involving 19 Partner Institutes from the EU, Turkey and China, the aim of which is to work together towards the minimization of the likelihood and consequences of future epidemic threats, through their expertise in the diagnosis and control of epidemic diseases of livestock. At the 4th Annual Meeting of the EPIZONE network, an interactive question session was run to elicit the opinions of delegates on future epidemic threats to the EU. The aim of the interactive session was to identify the most threatening viruses, both now and in the future, and to identify those tools which contribute most to prediction, prevention and control of future epidemics.

Delegates were asked a number of questions relating to six virus groups which were defined prior to the meeting. These were (1) influenza (2) bluetongue (BT) and African horse sickness (AHS) (3) African swine fever (ASF) (4) classical swine fever (CSF) (5) foot-and-mouth disease (FMD), and (6) West Nile fever (WNF), Rift Valley fever (RVF) and Crimean Congo haemorrhagic fever (CCHF). BT and AHS were grouped together, both being non-zoonotic arboviruses transmitted by Culicoides spp. whereas WNF, RVF and CCHF are all zoonotic arboviruses. The questions related to impact, likelihood, prevention and control and several options were given for each. Delegates were asked to consider the situation now and in the future (2020). Options were selected by delegates using a hand-held device and the collective results of all delegates were displayed instantaneously and discussed. After the meeting, results were investigated in more detail by stratifying according to various background questions.

In summary, delegates were of the opinion that FMD and influenza are currently the most threatening virus groups. They were also of the opinion that previous outbreaks of these viruses have resulted in changes by scientists, policy-makers and farmers in relation to both their practices and the way in which they view the factors contributing to epidemic threats. Although the future scenarios that delegates were asked to consider when thinking about future threats related to a relatively short time period of 10 years, overall, there was a change of opinion. In particular, delegates thought that influenza would be less of a threat and that the zoonotic arboviruses would be more of a threat in 2020 than now. CSF was considered least threatening both for now and in 2020. Differences in opinion were observed between delegates from industry and research, related to diseases worked on by the delegates, and the geographic region delegates came from.

When asked about the contribution of the EPIZONE Network in increasing our ability to predict, prevent and control future epidemics most delegates were positive, although non-EPIZONE delegates were slightly less convinced than EPIZONE delegates.

The results from the interactive session summarize the opinions of over 150 scientific experts on current and future epidemic threats. The virus group rankings should not be taken as definitive; rather the information could be used in conjunction with experimental and field data, by scientists, policy-makers and stakeholders when assessing and managing risks.

Acknowledgements: This project was funded by the EPIZONE Network of Excellence. We thank Margriet Vedder-Rootjes, Jitty Oosterga-Land, Dirk Pfeiffer and Cees van Hooff for their input into the interactive session.



ORAL: ASSESSING THE RISK OF H5N1 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS INFECTION IN POULTRY FROM MIGRATORY WILD BIRDS

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 VLA^1

Key words: qualitative risk assessment, wild birds, avian influenza virus, H5N1 HPAI

The objectives of the work presented here are to assess qualitatively the risks of release of H5N1 highly pathogenic avian influenza (HPAI) virus in Great Britain (GB), Italy and the Netherlands, and then to assess the risk of infection of poultry for GB only. The release, exposure and consequence framework according to OIE (2004) was followed. For the release assessment, 15 species of wild migratory waterfowl (including swans, gulls, ducks and geese) considered in the European Union as being higher risk species (HRS) with regard to H5N1 HPAI (CEC 2007) were studied. The release assessment was conducted at the level of the individual wild bird HRS taking into account ring recovery data from across the world, migration routes, times taken for migration and likelihood of survival of the infected bird and shedding of H5N1 HPAI virus on arrival. For the exposure assessment, five pathways from HRS birds to poultry were considered. These included both direct and indirect contact between poultry and the different wild bird HRS, consumption by poultry of HRS bird carcases and infection of other wildlife (bridging species), which subsequently contact poultry.

Preliminary results suggest differences exist in the probabilities of release of H5N1 HPAI through HRS birds between GB, Italy and the Netherlands, reflecting differences in the migration times, numbers of migrating birds and location of the country on migratory pathways. The probability of infection of poultry in GB by direct contact with 14 of the 15 HRS of waterfowl was estimated to be negligible, but found to be low for backyard poultry in contact with black-headed gulls. The 'bridging species' studied include pigeons, sparrows, mice, foxes, and mustelids.

The risk pathways are complex, particularly those involving bridging species, and there is considerable uncertainty in many components of the risk assessment and hence the predicted risks. For example, there is limited information on the probability that a HRS bird is infected in a particular region of the world given H5N1 HPAI is present in that region. The migration times vary both within and between wild bird species and there is little information on which to assess the level of contact between HRS birds and bridging species. However, by taking into account as much information as currently available this approach allows for a comparison of the risks of H5N1 HPAI infection through wild birds with other routes, namely imported poultry meat, live poultry and captive birds.

Acknowledgements: This work is funded through the EU 6th Framework Programme (FP6) project – FLUTEST, and the Veterinary Laboratories Agency (VLA).

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ORAL: RISK AND CONTROL OF AVIAN INFLUENZA, FOOT-AND-MOUTH DISEASE, AND CLASSICAL SWINE FEVER EPIDEMICS IN LIVESTOCK: COMPARISONS AND RULES OF THUMB

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 CVI^1

Key words: epidemic model, vaccination, FMD, CSF, HPAI

Large epidemics of highly-pathogenic avian influenza, foot-and-mouth disease and classical swine fever have struck livestock farming in a number of European countries in the past 10-15 years. Analyzing the epidemic data, mathematical models of the between-farm transmission dynamics have been constructed that are now being used to assess the expected impact of control strategies against future epidemics. We describe model transmission probabilities estimated from recent epidemics in The Netherlands of CSF (1997/1998), FMD (2001) and HPAI (2003) to make a comparison of these three diseases in terms of their between-farm transmission risks and the options for control by pre-emptive culling and emergency vaccination. The main epidemiological quantities determining the differences in transmission risk between these diseases are three: the distance-dependent transmission probability, the farm density distribution, and the generation time of infection. A further relevant quantity, determining in part how much emergency vaccination strategies can contribute to epidemic control, is the vaccine protection delay. Here we use these four quantities to compare the "controllability" of CSF, FMD and HPAI in The Netherlands. We formulate two rules of thumb for quantifying the potential of transmission and control.



ORAL: AN EPIDEMIC MODEL FOR THE WEST NILE VIRUS (WNV) DYNAMICS OF BIRDS, HORSES AND HUMANS

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Key words: Zoonosis, Arbovirus, Climate forcing, Temperature dependent parameters

Based on the Usutu virus model (Rubel et al., 2008; Brugger and Rubel, 2009), we developed an epidemic model for the simulation of the West Nile virus (WNV) dynamics of birds, horses and humans, which we apply to the Minneapolis metropolitan area (Minnesota, U.S.). The SEIR-type model comprises a total of 19 compartments, that are 4 compartments for mosquitoes and 5 compartments or health states for each of the 3 host species. It is the first WNV model that simulates the seasonal cycle by explicitly considering the environmental temperature. The latter determines model parameters responsible for the population dynamics of the mosquitoes and the extrinsic incubation period. Once initialized, our WNV model runs for the entire period 2002-2009, exclusively forced by environmental temperature. Simulated incidences are mainly determined by host and vector population dynamics, virus transmission and herd immunity, respectively. We adjusted our WNV model to fit monthly totals of reported bird, equine and human cases in the Minneapolis metropolitan area. From this process we estimated that the proportion of actually WNV-induced dead birds reported by the Centers for Disease Control and Prevention is about 0.8 %, whereas 7.3 % of equine and 10.7 % of human cases were reported. This is consistent with referenced expert opinions whereby about 10 % of equine and human cases are symptomatic (the other 90 % of asymptomatic cases are usually not reported). Despite the restricted completeness of surveillance data and field observations, all major peaks in the observed time series were caught by the simulations. Correlation coefficients between observed and simulated time series were R=0.75 for dead birds, R=0.96 for symptomatic equine cases and R=0.86 for human neuroinvasive cases, respectively (Laperriere et al., 2010).

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ORAL: HOW DOES INCREASING IMMUNITY CHANGE SPREAD KERNEL PARAMETERS IN SUBSEQUENT OUTBREAKS? – A SIMULATION STUDY ON BLUETONGUE VIRUS

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Technical University of Denmark¹

Key words: bluetongue; simulation; spread kernel; immunity

Modelling the spatial spread of vector borne diseases, one may choose methods ranging from statistic to process oriented. One often used statistic tool is the empirical spread kernel. An empiric spread kernel fitted to outbreak data provides hints on the spread mechanisms, and may provide a good estimate on how future epidemics could proceed under similar conditions. However, a number of variables influence the spread of vector borne diseases. If one of these changes significantly after an outbreak, it needs to be incorporated into the model to improve the prediction on future outbreaks. Examples of such changes are: vaccinations, acquired immunity, vector density and control, meteorological variations, wind pattern, and so on. Including more and more variables leads to a more process oriented model. A full process oriented approach simulates the movement of virus between vectors and host, describing density and motion of vectors/hosts, climatic variables, and so on will theoretically be able to describe an outbreak under any circumstances. It will most likely contain parameters not very well established, and is also very heavy in computer time. Nevertheless, we have tried to create a relatively detailed simulation spread model. And by using empirical spread kernels from past outbreaks we have fitted some of the more uncertain parameters for this case study.

A stochastic simulation model was developed for the spread of bluetongue virus. In the model hosts (cattle) and vectors (Culicoides) are distributed onto a grid representing farm/field quadrants of 1 hectare. Each quadrant has a host SEIR model (Susceptible Exposed Infectious Recovered) and a vector SEI model attached. Transmission of virus between hosts and vectors depend on many parameters most of which are temperature dependent. Spatial movement of virus between quadrants is modelled by local flight and wind spread of vectors.

The simulated spatial spread rate of virus is very dependent on movement parameters, but also the distribution and total numbers of hosts and vectors influenced the spread of virus. With empirical spread kernels from past outbreaks and known distributions of host animals, it was possible to fit parameter values of vector movement.

The final model including the fitted process based movement parameters is used to simulate e.g. 50% of cattle protected by acquired immunity after a first epidemic outbreak. We can then demonstrate how this changes the spread kernel for future outbreaks.



ORAL: BOVINE TUBERCULOSIS IN BELGIUM: EMPIRICAL APPROACH FOR A RISK BASED SURVEILLANCE PROGRAM

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VAR-CODA¹; Federal Agency for the Safety of the Food Chain²

Key words: Bovine, Tuberculosis, Risk based, Surveillance, Sensitivity

Since 2003, Belgium obtained the official free status (<0,1%) for bovine tuberculosis (bTB) by Commission Decision 2003/467/EC. EU Council Directive 64/432/CEE allows each MS which maintained the official free status for 5 consecutive years to review and adapt their national surveillance program.

In this context, a study has been carried out in order to evaluate different components of the current bTB surveillance program and to optimize this program. The main objective of this study was to install an improved risk- and target-based surveillance for bTB in Belgium.

Separate scenario trees were designed for each component of the surveillance program: i) slaughterhouse surveillance ii) testing at purchase surveillance iii) testing all imported animals surveillance and iv) tracing-on and tracing-back of outbreaks surveillance. Each scenario tree reassembled all steps from infection to detection. Surveillance data over the past 5 years have been collected, as well as animal population data, and data on animal movements.

Risk factors retained to be relevant were: zone, imports from countries at risk, status of the past, movement rate, herd type, and herd size.

A number of statistical methods were used to estimate in the most empirical manner the different values of risks nodes (animal population proportion, sampled population proportion, relative risks).

All these parameter estimates were fitted into the scenario tree model. Different stochastic simulations were carried out to measure the impact of modifications in one surveillance component relative to the other ones and also to determine the most optimal number of herds to be sampled and of animals to be sampled within each herd (ModelRisk).

Preliminary results illustrate that slaughterhouse surveillance should be maintained in contrary to the mandatory testing of all purchased animals of the current surveillance program that could be reduced. The mode across 10 000 iterations for the following 2 surveillance components was respectively for testing only purchased animals 0.87, and for testing only at slaughterhouse 0.92. Large variations around the mean values were observed. The sensitivity analysis showed that the most influencing parameter explaining this variability came from the uncertainty distribution around the diagnostic process parameters.

In the past, scenario trees have proved to be useful tools to enable the quantification of sensitivity of such a targeted surveillance and guarantee a certain level of confidence, providing that proper estimates of the main epidemiological parameters are available. Due to the availability of bTB surveillance data over the past 5 years, this study brought clarity on the current surveillance of bTB in Belgium. This study confirmed the current perception and revealed that more time and money should be allocated to certain populations at risk as well as certain components of the surveillance program.

Results of such models provide alternatives to policy makers to optimize the surveillance program, depending on the efficiency of detection, field work, and financial resources, such as required by the international standards.



ORAL: THE EU DISCONTOOLS PROJECT: DEVELOPING THE MOST EFFECTIVE TOOLS TO CONTROL INFECTIOUS ANIMAL DISEASES IN EUROPE

DELAVERGNE, MORGANE

In the Action Plan developed by the European Technology Platform for Global Animal Health (ETPGAH), disease prioritisation and gap analysis were seen to be fundamentally important and urgent actions to orientate future research in Europe. Following a call under FP 7, the DISCONTOOLS project was launched for a four year period in March, 2008.

The objective of DISCONTOOLS is to enable research to be optimised by public and private funders in a more effective manner to allow new and improved tools to be developed and delivered for the control of the major infectious diseases of animals including zoonoses.

DISCONTOOLS, as is the case with the ETPGAH, is stakeholder driven including representative organisations for academia, farmers, veterinarians, industry, CVO's and the International organisations including the OIE and FAO. The project is structured around 5 Work Packages with WP's 2 and 3 focusing on prioritisation and gap analysis respectively.

Within in the scope are 51 diseases (including those identified in the ETPGAH Action Plan). For each disease, a "Disease and Product analysis" document is developed collating key information to assist in the subsequent gap analysis and prioritisation work. To carry out the work, an 'Expert Group' has been established for each disease including expertise in epidemiology, immunology, pathology, an industry representative, a diagnostics expert and an individual with economic/trade expertise. On foot of the development of the D&P, the Expert Group then identifies gaps in diagnostics, vaccines and pharmaceuticals tools using a series of criteria for each subject area with an interpretation guide to hand to assist the process. Following gap analysis, the Expert Group then prioritises – again via a series of criteria with the assistance of an interpretation guide.

A website has been developed where the information is publicly available following an approval process involving WP 2 and 3 and the Project Management Board of DISCONTOOLS. The website includes a very flexible database which allows a multitude of ways to interrogate the data. As a means of keeping the data up to date and as a means of tapping into the global community, the website facilitates a type of 'Wikipedia' interaction with the comments being eventually assessed by the Expert Group and the data then updated via the normal approval process.

The DISCONTOLS website is of very considerable interest to all parties involved in funding research aimed at developing new or improved diagnostics, vaccines or pharmaceuticals. It provides a consensus view from all the stakeholders concerning the priorities. Being an interactive site, it will remain up to date and should become a key reference point. It is important that EPIZONE members provide input to the ongoing work of DISCONTOOLS to ensure that the most accurate information is contained, on an ongoing basis, on the DISCONTOOLS website.





Satellite symposium Bluetongue and other vector borne diseases 13th April, 2011





Keynote Lyle Petersen





KEYNOTE: GLOBAL EMERGENCE OF VIRAL VECTOR-BORNE DISEASES – WHY AND HOW?

PETERSEN, LYLE

The past three decades have witnessed a dramatic geographic expansion and increase in human incidence of many viral vector-borne diseases, most notably expansion of West Nile virus throughout the Americas, Japanese encephalitis virus in western parts of Asia, and Chikungunya virus in Africa, the Indian Ocean region, and parts of Southeast Asia and the Pacific. In addition, major Rift Valley fever epidemics have occurred in Africa, significant West Nile virus outbreaks have occurred occasionally in Europe and Russia, and tick-borne encephalitis and eastern equine encephalitis viruses have apparently expanded northwards. This lecture will explore the underlying complex sociological, environmental, virological, and climatic factors that may be responsible for these trends.





Epidemiology and risk assessment





ORAL: AHSV: CURRENT MOLECULAR-EPIDEMIOLOGICAL SITUATION IN SUB-SAHARAN AFRICA

BACHANEK-BANKOWSKA, KATARZYNA¹; MAAN, SUSHILA¹; BATTEN, CARRIE¹; SUTTEN, GEOFF²; OURA, CHRIS¹; MERTENS, PETER P.C.¹

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Key words: AHSV, epidemiology

African horse sickness virus (AHSV) represents a distinct virus species belonging to the genus Orbivirus within the family Reoviridae. AHSV is closely related to Bluetongue virus (BTV) the Orbivirus 'type species', with similar virus-particle morphology and replication strategies. Both viruses are transmitted between their equine or ruminant hosts primarily by biting midges (Culicoides spp.).

The Sahara represents a major geographical barrier helping to preventing the spread of African horse sickness (AHS) from Sub-Saharan Africa into Europe. However, the virus has previously caused significant fatalities and diseases outbreaks in horses beyond its current "established" endemic zone in Africa, persisting in newly affected areas (in India / Pakistan, and Europe) for a number of years. This suggests that environmental conditions and insect vectors suitable for successful transmission of AHSV exist in a much greater geographic area than that in which the virus currently occurs.

Since 1998 there have been multiple introductions of BTV beyond the 35oS and 40oN zone which was historically regarded as the BTV distribution area. Multiple BTV strains have spread to southern Europe from the North Africa, as well as via an unknown route directly from Sub-Saharan Africa into Northern Europe. These events, which have been linked to climate change, suggest that there is an increased risk that other insect transmitted viruses, including AHSV, could also be transmitted into Europe, with the potential to cause significant fatalities and major economic losses. A database for BTV nucleotide sequence has helped to determine the geographic origins of the different European field strains, in the identification of reassortant field/vaccine strains, and in the development of novel RT-PCR based diagnostic assays.

We report the initial development of an AHSV sequence database, based on strains from the Orbivirus reference collection held at IAH Pirbright. Sequences from reference strains and well documented field isolates of different AHSV serotypes, have provided a basis for the development and evaluation of novel RT-PCR based diagnostic-assays for AHSV. These real-time serotype-specific RT-PCR based assays, were used to identify multiple AHSV serotypes (AHSV-2, 4, 6, 8 and 9) as the cause of disease outbreaks in local horses in Ethiopia. Most of these serotypes had not previously been reported in the region. Multiple lineages of an AHSV serotype 9 were also identified circulating in non-vaccinated animals in the Gambia (see Abstract by Oura et al).

Current molecular-epidemiological data on the AHSV situation in Sub-Saharan Africa will be presented.



ORAL: EQUINE ENCEPHALOSIS AND AFRICAN HORSE SICKNESS IN THE GAMBIA

<u>OURA, CHRIS</u>¹; IVENS, PHILIP²; BANKOWSKA, KASIA¹; MAAN, NARENDER S.¹; BATTEN, CARRIE¹; JALLOW, DEMBA³; SAILLEAU, CORINNE⁴; ZIENTARA, STÉPHAN⁴; MAAN, SUSHILA¹; MERTENS, PETER P.C.¹

IAH¹; Royal Vet College²; Ministry of Agriculture³; AFSSA⁴

Key words: African Horse Sickness, Equine Encephalosis, Gambia

We describe the circulation of two Culicoides transmitted orbiviruses in equids in the Gambia. In response to a suspected African Horse Sickness (AHS) outbreak in the region in 2007 a vaccination programme was instigated. A monovalent live attenuated AHSV-9 vaccine from Senegal and a polyvalent vaccine from South Africa were administered to horses. In October 2009 a serological and virological survey for AHS and equine encephalosis (EE) was undertaken. In total 146 equids were sampled from 7 villages in the north / mid Gambia and 10 villages in southern Gambia. The clinical and vaccination history of each equid was recorded. The median age of the equids (103 horses, 43 Donkeys) was 7 years with a range of 1-20 years. Both groups contained predominantly unvaccinated animals. All horses (100%) were seropositive for Equine Encephalosis Virus (EEV). Six young donkeys were positive by polymerase chain reaction (RT-PCR) for EEV indicating circulation. EEV was isolated from two of these samples and the circulating serotype was identified by real-time RT-PCR as EEV-3 (Kaalplaas) (see abstract by N.S. Maan).

The majority of horses from both groups (96%) were also seropositive for AHS despite most being unvaccinated, with no history of clinical signs of AHS. Young horses (<3 years old) were shown to be seropositive for AHS from an early age and serum neutralisation assays showed that they had neutralising antibodies specific to AHSV-9. Eight young equids (<3 years) were positive for AHSV (by RT-PCR assay) and live AHSV was isolated from two of these horses indicating that the virus was circulating and was transmitted by the local midge population.

Serotype-specific RT-PCR assays targeting genome segment 2 (Seg-2 - the VP2 gene) identified AHSV serotype 9 circulating in The Gambia (see abstract by Bankowska). Sequence analysis confirmed this result with 100% identity to Seg-2 from the South African AHSV-9 reference strain (PAKrrah/09). We therefore concluded that the AHSV-9 live attenuated vaccine used in the region was circulating in the Gambia. However, further sequence analyses identified a mixture of different AHSV-9 strains in the Gambian AHSV-9 isolates. In addition to a vaccine derived strain (similar to the AHSV-9 reference strain), two other distinct Seg-2 sequences were identified, which although also belonging to AHSV-9, were not derived from the reference strain.

Sequence analysis (Seg-2) was also carried out on an AHSV-9 isolate that was isolated from horses in Senegal in 1998 that succumbed to fatal clinical signs of AHS immediately after entry into the country (Sailleau et al., 2000). This revealed only 95% identity to the 2009 Gambian AHSV-9, indicating that these isolates were not derived from the same source. We conclude that the 1998 AHSV-9 field strain was not derived from the AHSV reference strain / vaccine.

In conclusion, we have shown that EEV is endemic in The Gambia, infecting equids at an early age. The circulating serotype was identified as EEV-3 (Kaalplaas). We have also identified a strain of AHSV-9, which is likely to be derived from the live attenuated AHSV-9 vaccine that is circulating along with field strains within the equine population in the Gambia. This circulating AHSV-9 vaccine strain may to be 'immunising' equids from an early age in the Gambia, protecting from infection with more virulent field strains of the same serotype. Although this may have some beneficial effects, provided that the circulating vaccine-derived strain does not revert to virulence, there is clearly potential for genome segment reassortment with field strains that could cause reversion.



ORAL: MODELING PREVENTION AND CONTROL OF RIFT VALLEY FEVER EPIDEMICS IN THE NETHERLANDS

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 CVI^1

Key words: Rift valley fever, mathematical model, risk, spread, persistence, The Netherlands, Europe

Rift valley fever (RVF) is a vector-borne viral infectious disease of mammals, which was until a decade ago confined to Africa. Outbreaks on the Arabian peninsula gave rise to the fear of expansion to Europe. As shown by the establishment of bluetongue virus in North-western Europe, rapid invasion and persistence of a new vector-borne disease is possible.

RVF virus (RVFV) can infect a large range of animals, including many European livestock species and can cause severe disease in humans. In The Netherlands, several potential vector species are present. These can be grouped in vectors that transmit the virus after replication in the vector (mosquito-species Aedes spp. and Culex spp.), and mechanical vectors (stable fly, Stomoxys calcitrans). Some Aedes spp. are known to transmit the virus to their eggs.

Of major importance for the epidemiology of vector-borne diseases is the vector to host ratio. Vector abundance and host density, i.e. livestock density, are spatially heterogeneous, and hence the epidemiology of RVF is not equal for all areas in the Netherlands.

To determine the effect of interventions on the potential spread of RVF, we developed a mathematical dynamic model to determine the epidemic growth. The calculations are done for the vector abundance and host density at a five-by-five kilometre resolution. The model was parameterized based on a literature, livestock density data and vector population abundance estimates. The latter provided by Avia-GIS1 based on Dutch and Belgian vector counts that were linked to biotope, geographic and microclimatic data. Outcomes of the model will be used to create maps of the Netherlands showing the spatial heterogeneity in risk over the country.

The impact of three interventions, culling of animals, vaccination and vector control, were calculated. We determined the effect on the prevention of outbreaks in areas with different risks. Furthermore simulation of the course of the epidemic in exemplary area can give some crude insight in the effect of intervention during an ongoing outbreak.

Vaccination previous to introduction will reduce the number of susceptible animals in an area. The dilution effect of this intervention reduces the possibilities for an outbreak in this area. Model results indicate the minimum requirements of a vaccine to prevent spreading of RVFV in the Netherlands. The effect of vector control is modelled as a reduction of the vector population. Vector control can prevent major outbreaks, and furthermore shorten and reduce epidemics. Model results indicate the minimal requirements of vector control to prevent spreading of RVFV in the Netherlands.

Culling of animals will reduce the mean length of the infectious period of infected hosts and would be expected to limit the outbreak. However, we show that the culling of animals can have a two counter-acting effects, i.e. removal of infectious host and the increase of the vector-host ratio. If the vector population is not affected by culling of hosts, the vector-host ratio increases after culling, which increases the epidemic growth rate. When culling is fast enough, the outbreak can be shortened and prevented. However, when the speed of culling is too low, it can boost the epidemic. Model results indicate that culling can either boost the epidemic, or be an efficient control measure.

In conclusion, the model results indicate the minimum requirements for vaccines and vector control. Furthermore, the effectiveness of culling depends on the rate of culling.

¹www.avia-gis.com



ORAL: USUTU VIRUS IN ITALY, AN EMERGENCY OR A SILENT INFECTION?

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Key words: Genome sequencing, Italy, NS5, USUTU virus, wild birds

A two year study (2008-2009) was carried out to monitor the USUTU virus (USUV) circulation in Italy, Sentinel horses and chicken, wild birds and mosquitoes were sampled and tested for the presence of USUV and USUV antibodies within the WND National Surveillance plan. Seroconversion evidenced in sentinel animals proved that in these two years the virus has circulated in Tuscany, Emilia Romagna, Veneto and Friuli Venezia Giulia regions. In Veneto USUV caused severe disease in blackbirds which led to the death of about thousand birds. Eleven strains were detected in organs of 9 blackbirds (52.9%, 95% CI 30.9-74.0%) and two magpies (0.5%, 95% CI 0.15-1.8%) originated from Veneto and Emilia Romagna regions. USUV was also detected in a pool of C. pipiens caught in Tuscany. According to the alignment of the NS5 partial sequences, no differences between the Italian USUV strains isolated from Veneto, Friuli and Emilia Romagna regions were observed. The Italian North Eastern strain sequences were identical to those of the strain detected in the brain of a human patient and shared a high homology with those of isolates from Austria and Hungary. Conversely some minor differences were observed between these isolates and the USUV strain detected in C. pipiens caught in Tuscany. High degree of homology at both nucleotide and aminoacid level was also found when the full genome sequence of the Italian North Eastern isolate was compared with that of the strains circulating in Europe. The Italian USUV strain sequences exhibited 97% identity to the South African reference strain SAAR-1776. The deduced amino acid sequences of the Italian strain were 100% identical to the European strains except for 10 amino-acid conversely it showed 99% amino acid identity to the SAAR-1776 strain. This study proved that two strains of USUVs are likely to have circulated in Italy between 2008 and 2009. They have developed strategies of adaptation and evolution to spread into new areas and to become established.



ORAL: EUROWESTNILE: A EUROPEAN WNV COLLABORATIVE RESEARCH PROJECT

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Central Virology Laboratory¹; IZSLER²; VIRCELL, S.L.³; CISA-INIA⁴; AFSSA⁵; Fondazione Edmund Mach⁶; Veterinaermedizinische Universitaet Wien⁷; Istituto Superiore di Sanita⁸; Institut Pasteur de Paris⁹; Central Research Institute of Epidemiology¹⁰; Institut Pasteur de Dakar¹¹; Instituto de Salud Carlos III¹²; INGENASA¹³; Doñana Biological Station/ CSIC¹⁴

Key words: West Nile virus; Europe; FP7-Health 2010

West Nile (WNV) is one of the most evident examples of emerging/re-emerging pathogens one can put forward. West Nile disease is characterized by occasional virulent epidemic, epizootic and epornitic outbreaks. Despite intensive research done since its first appearance in the Americas in 1999, many aspects of its molecular biology, epidemiology, ecology, pathogenesis and life cycle are still poorly understood. Being a generalist pathogen par excellence, its eco-epidemiology is extraordinarily complex, involving hundreds of different vectors and hosts, which differ between locations.

In addition, as other RNA viruses lacking proofreading replication, its genome is highly variable and consequently of extraordinary plasticity. As a result, many WNV variants have evolved independently in different parts of the world. As the virus moves from one area to another, either by nature, through migrating birds, or by human influence (trade and/or other activities), different WNV variants (lineages) from different origins can coexist (and co-evolve) in a particular area. This is the case in Europe, where at least five out of a total of seven WNV genetic lineages have been identified to date. This situation is clearly different from that of North America. However, most studies on WNV currently come from the USA, biasing the knowledge available not only towards one of the lineages (lineage 1)-a serious bias with important consequences influencing, for instance, diagnostic methods- but also to the WNV ecology in hosts and vectors.

In the present EuroWestNile project (FP7-Health 2010) we will conduct comprehensive studies on the WNV situation in Europe and affected surrounding countries that accounting for the peculiarities of WNV eco-epidemiology in this region. Moreover we will strive to cover knowledge gaps regarding its ecology, epidemiology and pathogenesis, in order to better understand its different behaviour, as well as its current upsurging, in different parts of Europe and the Mediterranean. Finally, the project aims to develop new tools and strategies for research on treatment and prevention of WNV disease, as well as to produce new diagnostic methods, taking Euro-Mediterranean peculiarities into account.




Diagnostics and intervention





ORAL: EPITOPE MAPPING ON VIRAL PROTEIN 7 (VP7) OF BLUETONGUE VIRUS

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 CVI^1

Key words: Bluetongue DIVA VP7

Bluetongue virus (BTV), family Reoviridae, genus Orbivirus, contains ten double stranded RNA segments encoding at least ten viral proteins. The genus Orbivirus contains several serogroups of which BTVs and enzootic hemorrhagic disease viruses (EHDVs) are the most important serogroups in ruminants. These orbiviruses are arthropod-borne viruses; virus transmission to ruminants, including cattle, sheep, and goats, occurs by bites of species of Culicoides. Vaccination against several serotypes of BTV with inactivated vaccines and/or modified-live vaccines has been carried out for years all over the world, including in the European countries affected by BTV serotype 8. Serological monitoring is mainly performed by commercially available enzyme linked immunoperoxidase assays (ELISAs). These ELISAs are all based on the humoral response directed against the serogroup-specific structural protein VP7 of BTV. Seroconversion to BTV can be detected with these ELISAs irrespective to the involved serotype of the vaccine. However, the immune response by vaccination is indistinguishable from that by natural infection. The aim of this study is to determine the target of these commercial ELISAs. Further, possibilities will be investigated to modify this antigenic domain for development of DIVA* vaccine (*differentiating vaccinated from infected animals). Ideally, commercially available ELISAs are extensively validated and used, and could serve as DIVA-test to monitor BTV-circulation in a (partially) vaccinated population.

Based on the well-characterized 3-dimensional structure of the protein, VP7 protein can be divided into three parts; an N-terminal bottom domain, a centrally located top domain, and a C-terminal bottom domain. To study the immunogenicity of these different VP7-domains, BTV/EHDV chimeric VP7 genes were constructed by fusion PCR, and cloned in expression plasmids. EHDV/BTV chimeric VP7 proteins as well as VP7 of BTV and EHDV were transiently expressed in BSR-monolayers. VP7 proteins were detected with sera against BTV and EHDV in an immunoperoxidase monolayer assay (IPMA). Results showed no cross reactivity between EHDV and BTV. The top domain is by far the most antigenic part of VP7 of BTV, since only immunostaining was observed in the presence of the respective top domain. In addition, a set of monoclonal antibodies (MAbs) that have been raised against BTV and directed to VP7, all bind to epitopes located in the top domain. Finally, a few of these epitopes could be mapped by comparison of top domains of BTV-strains showing different IPMA-results with the respective MAbs. Future research will be focused on this part of VP7 (fine mapping). The scientific challenge will be to rescue virus (van Gennip et al., 2010, 2011) in which modification and maintenance of functionality of VP7 is balanced in order to develop DIVAvaccines.

References van Gennip et al., 2010, BMC Vi

van Gennip et al., 2010. BMC Virology Journal 7, 261-267. van Gennip et al., 2011 in prep., and presentation/poster.



ORAL: INTERACTION OF BLUETONGUE VIRUS WITH THE INNATE ANTIVIRAL RESPONSE

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AFSSA¹

Key words: BTV, innate immunity, interferon

The innate immune response is the first line of defense against viral infection. However, most of viruses have evolved diverse strategies to escape this response. Orbiviruses, especially BTV (Bluetongue virus), EHDV (Epizootic hemorrhagic disease of deer virus) and AHSV (African horse sickness virus), are major animal health concerns transmitted through the bites of Culicoides vectors. Due to the lack of reverse genetics model for these agents until recently, the molecular mechanisms that govern the viral pathogenesis have remained poorly investigated. In 70's and 80's, some works showed that BTV infection triggers the production of type-I interferon (IFN-I), a pivotal mediator of the innate immunity, and that genomic dsRNA is a strong IFN-I inducer. However, cellular pathways involved in the IFN-I synthesis following BTV infection remain unknown. Most of viruses have evolved diverse strategies to escape the IFN-I response but nothing is known on the ability of BTV to counteract the innate antiviral response.

Using a luciferase reporter assay, we recently showed that the RIG-I-like receptor pathway is involved in the innate immune response in BTV infected cells. We also demonstrated that BTV serotype 8 can dampen the type-I interferon response. We are trying now to identify viral components involved in this inhibition and to explore its possible link with viral pathogenesis. Afterwards these results will be confirmed at the virus level by introducing specific mutations within the dedicated BTV-8 infectious clone. We also aim at evaluating the conserved feature of these functions by performing similar experimental study with EHDV and AHSV.



ORAL: REVIEW OF THE ORAL SUSCEPTIBILITY RESULTS FOR DETERMINING VECTOR STATUS OF CULICOIDES SSP. AS TRANSMITTERS OF BTV IN EUROPE AND SOUTH AFRICA

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Key words: Oral susceptibility, Vector competence, BTV European strains, BTV African Strains

Oral susceptibility tests are one of the main tools that are being used to date to demonstrate the ability of the different Culicoides species to transmit the Bluetongue virus. The oral susceptibility studies of Du Toit (1944) in South Africa, which for the first time clearly demonstrated the role of Culicoides midges in the transmission of orbiviruses, were subsequently confirmed elsewhere in the world i.e. in North America, Australia, and England. In 1991 in South Africa field-collected Culicoides (Avaritia) imicola Kieffer was fed on sheep blood containing either BTV-3, -6. After an extrinsic incubation period of 10 days, the rates of infections in C. imicola for BTV-3 and -6 were established at 31% and 24%, respectively. Oral susceptibility studies involving 29 different BTV serotypes/isolates and field collected Culicoides species indicated significant differences between the various isolates used and Culicoides (Avaritia) bolitinos Meiswinkel be significantly more susceptible to oral infection with most of the BTV serotypes/isolates than C. imicola. It also indicated that laboratory attenuation does not reduce their ability to infect competent Culicoides species and may even lead to enhanced replication in the vector. Oral susceptibility studies in South Africa show that BTV to replicate in Culicoides species of six different subgenera thereby implicating multi-vector potential for the transmission of these viruses. This multi-vector potential for the transmission of BTV is supported by the isolation of BTV from various field collected Culicoides species in South Africa as well as Europe.

Studies performed with Spanish strains belonging to three different BTV serotypes (BTV-1, -2, and -4) assayed in South African Culicoides population (del Rio et al unpublished data), demonstrated that Culicoides imicola can become infected at the same rate than those found by Venter et al when testing the African strains of the same serotypes. However, Venter et al., (2010) demonstrated that the susceptibility of South African C. imicola to a series of strains of BTV-8 from both Europe (Spain, Belgium and the Netherlands) and Africa was extremely low. On the other hand, Carpenter et al also demonstrated that Palaearctic species C. obsoletus and C. scoticus were susceptible to infection with BTV-8 from the Netherlands using pledget feeding technique.

Despite some of the shortcomings of the oral susceptibility technique (i.e.: adaptation of virus strains to cells passages), it is still the method which represents greater similarity with field BTV transmission by Culicoides, since it demonstrates the replication of the virus during the extrinsic incubation period into the assayed midges.

The results obtained to date about oral infection studies indicate that susceptibility to BTV may indeed be widespread in the genus Culicoides. The data obtained during these last years seem to indicate that the susceptibility and vector competences of some of the Palaearctic Culicoides species may be equal to or even higher than that of the proven vector C. imicola.



ORAL: EFFICACY OF BTVPUR ALSAP® 8 IN LAMBS, IN THE FACE OF MATERNALLY DERIVED ANTIBODIES

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MERIAL S.A.S.¹; INRA²

Key words: BTV8, sheep, vaccine, efficacy, MDA

Introduction

Following the recent BTV-8 epizooty, populations of domestic ruminants have been widely vaccinated against BTV-8 across Europe. Consequently, most young ruminants are now passively immunized against BTV-8 through maternally derived antibodies (MDA).

There has been an ongoing debate on the effect of MDA at protecting newborns or on their possible interference with vaccination.

To study the impact of MDA on BTV-8 vaccination, we designed and conducted a vaccination/challenge study in lambs born from immune ewes. We describe here the serological, clinical and virological evaluation of vaccination programs at different ages, in lambs with MDA, using BTVPUR AlSap® 8 (Merial).

From the results, an optimal age at vaccination with BTVPUR AlSap® 8 is proposed, taking into account the increase in susceptibility to BTV-8 accompanying the decline of MDA and the interference of MDA on vaccination.

Material and methods

Forty, 1-month old lambs, born from immune ewes and sero-positive to BTV-8 were randomly allocated to 5 groups of 8 lambs. Each group was subcutaneously vaccinated with a single 1 mL dose of BTVPUR AlSap® 8 (commercial batch) at the age of : Group 1 : 1.5 month ; Group 2 : 2.5 months ; Group 3 : 3.5 months ; Group 4 : 4.5 months ; Group 5 : Not vaccinated.

At 5.5 months of age, all lambs were challenged with a virulent BTV-8. All sheep were then monitored daily for rectal temperature and clinical signs from 5 to 14 days after challenge. Furthermore, all sheep were monitored for viraemia (validated qRT-PCR) 0, 5, 7, 9, 12 and 14 days after challenge.

Results

All controls, developed fever, clinical signs typical of BTV infection and all were viraemic at high titre, at all time points after the challenge.

Four of the 7 lambs of group 1 were fully protected (clinical signs and viraemia) while the three others were not protected.

In the groups vaccinated at 2.5 or 3.5 months, 87 % and 100 % of the lambs respectively were protected both clinically and virologically.

Among the lambs vaccinated at 4.5 months, 2 out of 7 were not protected. Globally, in the groups (G2, G3, G4) vaccinated at 2.5 months or later, 87% of the lambs were protected clinically and virologically against the challenge.

The study results have shown that:

- all lambs were susceptible to challenge at 5.5 months, and likely fully receptive to vaccination
- a large proportion (57 %) of the lambs were receptive to vaccination at 1.5 months

- most (87 %) of the lambs vaccinated at 2.5 months or later were fully protected - MDA may possibly persist up to 4.5 months and may interfere with vaccination in a small proportion of the lambs, while at that age, most lambs have become again susceptible again to BTV and receptive to vaccination.

Conclusion

From the study results, it is proposed that vaccination of lambs with MDA at 2.5 months or later would represent an appropriate balance, at flock level, between susceptibility to BTV of the young stock and interference of MDA, keeping in mind that at that age, a small proportion of the lambs may not be fully protected.



ORAL: EPIZONE: EPIZOOTIC HEMORRHAGIC DISEASE VIRUS IN EUROPEAN CATTLE AND SHEEP: VIRULENCE, DURATION OF VIREMIA AND CROSS-PROTECTIVE EFFECT OF PREVIOUS BLUETONGUE VIRUS INFECTION

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FLI¹; IAH²; INSTITUTO G. CAPORALE³

Key words: Arboviruses, BTV, EHDV, Emerging diseases, Experimental infection

Epizootic hemorrhagic disease is an arbovirus disease of ruminants caused by Epizootic hemorrhagic disease virus (EHDV), an Orbivirus (family Reoviridae) that is closely related to Bluetongue virus (BTV). EHDV infection can cause severe disease and death in deer and, less frequently, a bluetongue-like illness in cattle. Sheep can be infected experimentally but rarely develop disease. EHDV regularly occurs in North America, Australia, Asia and Africa. Recently clinical cases due to EHDV infection have been reported in cattle in the Mediterranean basin (Northern Africa, Turkey and Israel). EHDV is transmitted by insect vectors, usually Culicoides midges. Studies on the length of viremia in deer and cattle showed that they can remain viremic for periods exceeding 50 days, but there is no data on the length of the viremia in sheep.

The recent BTV epizootics have demonstrated that orbiviruses can suddenly appear in Europe, spread quickly and cause severe disease in unprotected livestock. There now is widespread immunity to BTV after natural infection and mass vaccination campaigns, but no EHDV vaccines are available.

This study addresses several questions:

- Can EHDV infection cause disease in European cattle and sheep?
- Are the commercially available real-time RT-PCR and ELISA kits fit for purpose?
- How long can infectious virus and viral genome be detected in blood?
- Is there a cross-protective effect of previous BTV infection?

Four BTV-naïve Holstein cattle and four East Frisian sheep were experimentally infected with a 2006 Israeli isolate of EHDV-7. Four cattle and four sheep that had been infected with BTV-6 six weeks before were included in the experiment to examine the influence of previous exposure to another orbivirus.

After infection, the animals are clinically monitored. Blood samples are taken daily for two weeks, and three times per week for the next six weeks. Virus replication is measured by realtime RT-PCR. The duration of viremia will be determined by virus isolation on Vero and KC cells, inoculation of embryonated chicken eggs and type I interferon receptor deficient mice. Serological data is collected by blocking ELISA and serum neutralization tests.

In a close cooperation between the EPIZONE partner institutes FLI and IAH and the istituto G. Caporale, samples collected in the animal experiment at the FLI will be analysed at three European centers for orbivirus research. The results of the animal experiment and their significance for animal disease control in Europe will be discussed at the conference.





Keynote Piet van Rijn





KEYNOTE: EPIZONE AND THE BTV-8 EPISODE, REMARKABLE TIMING AND OPPORTUNITIES!!

VAN RIJN, PIET A.

In the 5-years of EPIZONE, many European countries have suffered from an outbreak of bluetongue virus serotype 8 (BTV-8). In this BTV-8 episode, research on bluetongue was significantly accelerated. Virological and serological diagnostics were developed and/or improved. Established assays were harmonised by efforts of EPIZONE and organized by the Community Reference Institute and others. New assays, like ELISAs for detection of BTVantibodies in milk, and serotype-specific PCR-assays have reached the market and are thus commercially available. New assays to identify circulating viruses in more detail are under development. Several vectors, species of Culicoides and natural vector of BTV, were identified for transmission of BTV in the moderate climate of north-western Europe. On the other hand, the route of introduction of BTV-8 has been never discovered, and only little progress has been reported to unravel the mechanisms of the fast spread of BTV by these European tiny insects. Indeed young scientists with interest in entomology are educated, but the number of experienced entomologists is still very limited. A provisional vaccine for BTV-8 became available after two seasons of BTV-8, which has drastically reduced the number of infections. In general, this episode has also shown that awareness and preparedness should be improved. Collaboration and established networks like EPIZONE are tremendously important to combat diseases, in particularly vector-borne diseases, threating livestock and human health.

Southern European countries at the Mediterranean Basin are suffering from Bluetongue caused by several serotypes, 1, 2, 4, 8, 9, and 16, for more than 10 years. In August 2006, BTV-8, a serotype not previously circulating in Europe, was detected in north-western Europe. Most likely, the introduction had occurred a few months ago in Belgium, coincidently in the same time that EPIZONE was officially launched in Brussels. A few countries were affected by this BTV-serotype in 2006, but after a mild winter Bluetongue reappeared. Infections were reported at many places and in the same time, indicating that BTV-8 had easily 'overwintered'. A devastating season followed in which thousands of holdings became affected. The affected area expanded in all directions, including northwards and the overseas United Kingdom. Meanwhile, several companies started the development of an inactivated BTV-8 vaccine. After this devastating year, again a mild winter followed. In May 2008, provisionally inactivated vaccines were launched. Many countries promptly started voluntary or obligatory vaccination of ruminants. Reported cases dropped drastically in 2008, due to these vaccination campaigns, and likely by the high percentage of natural immunity in heavily affected areas. By testing for international trade purposes, surveillance, or increased alertness, the temporary presence of vaccine-related viruses and a new BTV-serotype were detected in this period. The next seasons were followed by much colder winters in N-W Europe. This likely decreased the chance on overwintering of BTV-8, but the enthusiasm of holders to vaccinate decreased. Anyway, after the first year of mass vaccination, it became remarkable 'quiet' and in some countries even completely 'silent'. In conclusion, the episode of BTV-8 has shown that unfortunate introduction of a vector-borne disease in N-W Europe could result in a huge outbreak with enormous impact on the economics and society. It seems to be that vaccination is the most effective measure to control this vector-borne disease.

Bluetongue is a disease in all ruminants caused by members of the BTV-serogroup within the genus *Orbivirus* of the family of *Reoviridae*. At least 24 BTV-serotypes have been recognized, and new serotypes are recently proposed, mainly based on genetic data. BTV-infections can run from completely subclinical to very severe resulting in death. This course is dependent on the isolate or strain, but is irrespective from the serotype. Virus transmission between ruminants, including cattle, sheep, and goats, occurs by bites of species of *Culicoides*, but only a few species (competent vectors) seems to be effective in transmission (spread). Several alternative routes of transmission have been described. These are not significant in endemic areas, but could play a role in transmission over long distances to BTV-free areas



(introduction), and/or after a long period without virus circulation (overwintering). Bluetongue is associated with the presence of competent vectors. Consequently, Bluetongue shows a seasonality and the affected area can vary dramatically over the years.

BTV-8 (IAH collection nr. BTV-8 NET2006/04) has caused a huge outbreak in N-W Europe, and is different from most strains so far. Fast spread by endemic *Culicoides* species in moderate climate conditions, more severe clinical signs in cattle, and transplacental transmission are a few examples. Orbivirological research has a long record; several excellent research groups have studied BTV and related orbiviruses for decades. Bluetongue virus (BTV) contains ten double stranded RNA segments encoding at least ten proteins. Seven are structural proteins, and can be found in the virus particle. Three of these, VP1, VP4 and VP6, contain enzymatic activities involved in replication and transcription of the BTV-genome segments. Structural proteins, VP3, VP7, VP2 and VP5, form the rigid virus particle. In addition, three nonstructural viral proteins, NS1, NS2, and NS3/3a cannot be detected in the virus particle, but are expressed in the cytoplasm of infected insect– or ruminant cells, and interact with the viral RNA and/or with cellular components.

In the recent five years, the 5-years of EPIZONE, and the BTV-8 episode, molecular orbivirology has made a breakthrough by the development of reverse genetics for BTV. This technology opens the way to generate all kind of reassortants and mutants of BTV. Unknown functions of viral proteins can be identified and will be studied in more detail. For the first time, functions of viral proteins as well as unique viral mechanisms and interactions with cellular proteins can be studied in the infected host or vector. Additionally, better and cost-effective vaccines could be developed to control outbreaks of Bluetongue. Expectedly, this breakthrough will also accelerate research on other orbiviruses of concern, like African horse sickness virus. At present, only a small step has made to explore the opportunities of this technology in orbivirus research. A few examples of results, like reassortants, serotyped virus, and mutations in structural and nonstructural proteins, are presented by posters. Here, some of the opportunities for future research on this intriguing vector-borne disease will be presented and discussed.



Epidemiology and risk assessment





ORAL: WHOLE-GENOME ANALYSIS OF BTV STRAINS FROM MOROCCO REVEALS THE EMERGENCE OF A NOVEL REASSORTANT VIRUS – A NEW THREAT FOR EUROPE?

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Key words: Bluetongue virus, Reassortment, BTV-4, Morocco, sequence analysis

Bluetongue virus is the type species, genus Orbivirus, family Reoviridae. Bluetongue viruses (BTVs) can infect most ruminants, causing severe haemorrhagic disease called 'bluetongue' in sheep. BTVs are transmitted between their ruminant hosts by certain species of Culicoides (biting midges). The BTV genome is composed of ten linear dsRNA genome segments, encoding 7 structural and 3 non-structural proteins.

The recent emergence of bluetongue virus across Europe has involved multiple strains, belonging to both eastern (BTV-1, 9, 16) and western topotypes (BTV-1, 2, 4, 6, 8, 11 and 25), events that have been linked to climate change in the region. BTV-8 arrived in the Netherlands in 2006. It survived the cold northern European winter, before re-emerging with greatly increased intensity in 2007, killing many thousands of animals (mainly sheep) and spreading to almost the whole of Europe. However, massive vaccination campaigns implemented against both BTV-8 and BTV-1, using inactivated tissue culture grown virus as vaccine antigens, have been very effective.

BTV-4 arrived in Morocco in September 2004, spreading to the region of Spain and Portugal where Culicoides imicola is distributed. BTV-1 was also detected in Morocco during 2006, spreading to Algeria, Tunisia and Italy, then to Spain, Portugal (possibly via windborne midges) and as far as the north east coast of France (in 2008). This strain of BTV-1 can therefore be distributed by the northern European Culicoides species. However, vaccination campaigns in Spain, Portugal and France, using inactivated vaccines against BTV-1 and BTV-4, also appear to have been very effective. BTV-1 and BTV-4 re-emerged in Morocco during 2009 (with BTV-4 as the dominant serotype) spreading into Algeria. It was suggested that the pathogenicity of the novel BTV-4 strain was more 'comparable' to BTV-1 than to the previous 'less-virulent' BTV-4 strain from 2004.

The arrival and co-circulation of multiple BTV strains in Europe and North Africa has provided many opportunities for these viruses to exchange genome segments, potentially creating progeny virus strains with novel biological characteristics. The emergence of novel (reassorted) virus genotypes during an outbreak, suggests that they must have some selective advantage in the local ecosystem compared to the original parental strains. Full genome sequencing and phylogenetic studies of BTV-1 (2006) and BTV-4 (2004) in Morocco, show that these original viruses reassorted, generating the more recent strain of BTV-4 (MOR2009/09).

BTV-4 was very recently also been detected in a sentinel herd in southern Spain (OIE report 01/10/2010 - http://www.oie.int/wahis/public.php?page=single_report&pop=1&reportd=9773) suggesting that it arrived from Morocco, and may therefore represent the reassortant virus. The reassortant BTV-4 strain is likely to have acquired some characteristics from the parental BTV-1, which may include the ability to be transmitted by northern European Culicoides species, and it could potentially represent a further new threat to the region. These analyses have demonstrate the utility of whole BTV-genome analyses, although additional data will be required to fully elucidate the evolutionary mechanisms and epidemiological dynamics of the new virus.



ORAL: TRANSMISSION OF BLUETONGUE VIRUS SEROTYPE 8 THROUGH ARTIFICIAL INSEMINATION USING SEMEN FROM NATURALLY INFECTED BULLS

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Key words: bluetongue, BTV-8, transmission, semen

Right from the start of the bluetongue (BT) epidemic in 2006 in Central-Western Europe it became clear that this BT virus, identified as serotype 8 (BTV-8), had some atypical features. Opposite to other wild type BTV serotypes, BTV-8 caused clinical signs in cattle and foetal infection in pregnant ewes and cows. Moreover, as a consequence of natural BTV-8 infection, bulls were found shedding infectious BTV-8 in their semen. The presence of BTV-8 in semen was demonstrated by real-time RT-PCR (RT-qPCR) and virus isolation. So far, no evidence of seminal transmission of a BTV field strain was published. A study was set up in order to support the national authorities in the discussion and risk analysis related to trade in commercial semen. The objective of the current study was to investigate the possibility of transmission of BTV-8 to the recipient cow after artificial insemination (AI) using semen from naturally infected bulls and to check the effect on the offspring. Eighteen BTV-free heifers were artificially inseminated using four different batches of semen from which BTV-8 was isolated (3.5 to 4.7 TCID50 per straw). The heifers were successfully bred and eight became infected at different time points after AI. These heifers developed BT antibodies and BTV-8 RNA was detectable for a short or long period of time in their blood. Until now, five of the infected heifers aborted. The current study is still running, but so far the results provide evidence of BTV-8 being transmitted in cattle by artificial insemination.



ORAL: RO-MODELING AS A TOOL FOR EARLY WARNING AND SURVEILLANCE OF EXOTIC VECTOR BORNE DISEASES IN DENMARK

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Key words: Vector borne; Bluetongue; Risk assessment; Risk based surveillance; Early warning

Modelling the potential transmission intensity of insect borne diseases with climate driven R0 process models is frequently used to assess the potential for veterinary and human infections to become established in non endemic areas. Models are often based on mean temperatures of an arbitrary time period e.g. a monthly temperature mean. Temperature decreases with latitude, and in the Nordic countries periods of suitable temperatures, the windows of opportunity for transmission, may be very short and only appear in odd years. While average monthly temperatures are likely to be suitable for predicting permanent establishment of presently exotic diseases, mean temperatures may not predict the true potential for local spread and limited outbreaks resulting from accidental introductions in years with temporary periods of warm weather.

We present a system for continuous risk assessment of potential local spread of exotic insect borne diseases of veterinary and human importance. R0 models for various vector borne diseases are continuously updated with spatial temperature data to quantify the present risk of autochthonous cases (R0>0) and the present risk of epidemics (R0>1) in case an infected vector or host are introduced to the area.

The continuously updated risk assessment maps functions as an early warning system allowing authorities and industry to increase awareness and preventive measures when R0 raises above the level of 'no possible transmission' and target active serological surveillance to these limited periods of potential risk, thus dramatically reducing the number of samples collected and analysed. The risk estimated from the R0 modelling may be combined with the risk of introduction from neighbouring countries and trading partners to generate a truly risk based surveillance system for insect borne diseases.

R0 models for many vector borne diseases are simple and the available estimates of model parameters like vector densities and survival rates may be uncertain. The quantitative value of R0 estimated from such models is therefore likely to deviate from the true R0. However assuming the models are qualitatively able to rank the estimated R0 correctly, a period resulting in a relatively high estimated R0 will also be a period with a relatively high true R0. This allows the estimated R0 to be used for targeted surveillance by focussing the surveillance on periods and areas with high R0 estimates even if the actual value of these estimates are difficult to interpret. Furthermore running R0 models on historic outbreaks in Europe may be used to fit estimates for R0 for these data. When comparing the model R0 to the observed value of R0 a correction factor is obtained that may be used to adjust the model estimates in Denmark, and thus allowing a more quantitative interpretation of the estimated R0.

We here demonstrate the system for bluetongue using 2008 climate data and compare the predicted R0 with the actual spread of bluetongue in Scandinavia in 2008.



ORAL: ECONOMIC CONSEQUENCES OF BLUETONGUE DISEASE, SEROTYPE 8, IN GERMANY

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Key words: bluetongue disease, economic consequences, Germany, vaccination, eradication

In August 2006, bluetongue virus serotype 8 (BTV-8) was introduced into Central Europe. The virus hit an area with a high population density of BTV-naïve ruminants, suitable vectors (Culicoides spp.) and climatic conditions favourable for virogenesis and transmission. In 2006 and 2007 the disease spread over wide parts of western Germany and had a high economic impact on sheep and cattle farms. To reduce animal losses, avoid clinical symptoms and stop the further spread of the disease, Germany decided to implement a vaccination program with monovalent, inactivated vaccines against BTV-8 in May 2008. In 2008 and 2009, vaccination was obligatory for cattle, sheep and goats; since 2010, vaccination against BTV-8 is optional.

An economic analysis of the BTV-8 epidemic was performed based on data provided by the Federal States, the animal health services, the animal compensation funds (Tierseuchenkassen) of the Federal States, the German animal disease notification system (Tierseuchennachrichten, TSN), and the Federal Statistical Office for the years 2006-2010.

Bluetongue disease had a high economic impact on the cattle and sheep industry in Germany. In 2007, the costs for a total of 43,000 animals (33,233 sheep, 10,240 cattle and 102 goats) were claimed from the animal compensation funds. On the basis of the animal market values, this corresponded to more than 20 million Euros, including the disposal of the animal carcasses. Direct costs were incurred by BTV-8 infected farms due to reduced milk and meat production through mortality and morbidity, palliative veterinary care costs and fertility problems. Indirect costs resulted from vaccination, movement restrictions, and adapted export certificates. Further indirect costs like reduced sales opportunities due to image loss as well as costs for disease control (e.g. surveillance and monitoring to prevent further BTV-8 expansion, administration etc.) were not taken into account because they could not be quantified.

The total costs of BTV-8 were estimated at around 110 million Euros in 2007. In 2008 the costs for the eradication increased to more than 200 Million Euros and decreased after 2008 to about 100 million Euros in 2009 and 25 million Euros in 2010. Since 2008, the vaccination costs were the most expensive entry. In 2008, vaccination cost more than 160 million Euros, in 2009 about 100 million Euros and in 2010 about 25 million Euros. The economic impact of different control strategies will be discussed.



ORAL: BREEDING SITES OF BLUETONGUE VIRUS VECTORS IN BELGIAN COWSHED

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Key words: Culicoides, Bluetongue, Breeding site, Cowshed, C. obsoletus/scoticus complex

Bluetongue is an emerging disease of ruminants that was reported in August 2006 in northern Europe. In 2007 and 2008, BTV serotype 8 continued its spread across Europe and showed virulence in France. This observation indicates possible overwintering of the vector from year to year. The biting midges responsible for transmission of BTV in northern Europe belong to the genus Culicoides, but only few species are vectors of this virus. Breeding sites of bluetongue vector species have been found near farms (e.g. silage residues) and in neighboring meadows (e.g. overwintering cattle dung) but never inside sheds.

We conducted a study on five cattle farms in Belgium during February–October 2008. Three samplings were performed and each soil sample collected inside cowsheds was incubated to enable adult midges to emerge. Among 15 soil biotopes sampled, only one showed the emergence of adult Culicoides biting midges: dried dung adhering to walls inside animal enclosures and resulting to the partial removal of animal litter. It was a breeding site for the C. obsoletus/scoticus complex. Physico-chemical characteristics showed that midges of this complex are more prevalent in soil samples with a high carbon:nitrogen (C:N) index. So Culicoides biting midges are able to complete their life cycle in animal enclosures.

We identified a breeding site for the primary BTV vector in a cowshed in northern Europe. These observations could explain the persistence of BTV from year to year despite fairly harsh winters.

Hygienic measures on farms - such as removal of residual animal feed and feces and of material from silage structures and sheds - could reduce midge populations and improve efficacy of vaccination campaigns against BT in Europe.



ORAL: SPATIAL MODELS FOR THE DISTRIBUTION OF CULICOIDES ON A LOCAL SCALE

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Key words: bluetongue virus, vectors, Culicoides, distribution, spatial modelling

Introduction

Since 2006, bluetongue virus (BTV) has been present in the temperate region of Europe. Apparently, BTV is able to utilize new vectors in this region. The main vector groups appear to be the Culicoides obsoletus and the C. pulicaris vector groups. In order to predict the spread of BTV we need to able to predict the spread of the vectors. The first step in developing a vector dispersal model is to describe the distribution of the vectors. In this study we examine relevant spatial covariates and build a prediction model based on empirical data.

Method

The battery-operated CDC mini UV-light trap was chosen for estimating vector abundance. Vectors were caught in 50 light traps set up on a field in Denmark in a 50 by 50 meter grid. For each trap location, the following spatial covariates were established: 1) Distance to nearest breeding site (of the C. pulicaris group) 2) The effect of windbreak cover from a tree line surrounding the field 3) Attraction of vectors to host animals. During the study period, temperature, wind speed and wind direction was also measured and included as non-spatial covariates. The effect of windbreak cover and the attraction of vectors to the host animals were modeled to give maximum effect if the wind was perpendicular to the windbreaks and then gradually decreasing with changing wind direction. If the wind was parallel to or blew away from the field, no effect of the windbreaks was assigned. The closer to the tree line, or the host animals, the higher the effects. The interactions between windbreak cover and wind speed, windbreak cover and host animals, and the squared temperature, were also tested for significance. Resulting coefficients were adjusted for spatial autocorrelation with neighboring traps, using a CAR model.

Data

Eight days were analyzed, with a total count of 5180 female vectors of both species groups together. Huge differences in the numbers of daily vector catch was observed. As a consequence, data were log transformed. A linear mixed model with random effect of catch day was used to build a prediction model.

Results

For the C. pulicaris group, distance to breeding sites and host animals was not statistically significant, neither was temperature. A negative effect was found for wind speed and a positive effect of windbreak cover. The spatial autocorrelation was highly significant. For the C. obsoletus, no spatial covariates were identified, but there was a significant negative coefficient for wind speed. The spatial autocorrelation was also found to be highly significant.

Discussion

The results of this study show that it is important to analyze the vector groups separately. The lack of significance of temperature could be explained by the relatively high temperatures during the study period (12-20 degrees C). For both species groups the non-spatial wind speed was found significant. This was expected as this pattern is general for small flying insects. There was no effect of the distance to breeding sites, which may be because the vectors avoided the open areas where the breeding sites were. The distance to host animals was not significant. This was surprising and contrary to the general agreement that traps should be placed near host animals. Apparently, this was not the case on the scale investigated in this study. The significant effect of windbreaks on the C. pulicaris group but not the C. obsoletus group may indicate that the former group of larger species is strong enough to benefit from the lower wind speeds provided by windbreak cover.



ORAL: MODELLING THE DISTRIBUTION OF CULICOIDES BLUETONGUE VECTORS IN NORTH WALES

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Key words: bluetongue, Culicoides, satellite-derived, abundance, ecology

Bluetongue (BT) serotype-8 emerged in northern Europe in 2006 and the UK initiated a vaccination campaign in 2007. With Wales accounting for 15% of all sheep within the EU community, incursions of BT could cause devastating production losses. Although all Culicoides species share the same basic habitat requirements, they differ in the extent to which environmental factors affect their distribution and abundance.

This study aims to identify determinants of local scale Culicoides abundance in the Bala region of north Wales. The distribution and abundance of BT vectors and other Culicoides species are modelled in relation to satellite-derived variables, and host and environmental variables at the 1km scale. Specific objectives included to: 1) determine local-scale predictors of abundance; 2) build a good model for C. obsoletus (main UK vectors); 3) determine whether predictors can be generalised across species with particular ecological characteristics/groups; 4) investigate the use of free high-resolution satellite imagery at a local spatial scale.

Culicoides surveillance was carried out on 35 farms around Bala over 12 nights in July 2008. Environmental variables, including the presence of breeding sites and water sources, as well as on-farm host factors (animal numbers and insecticide use), were assessed using an on-farm questionnaire. Seventy remotely-sensed MODIS variables, from the NASA Terra satellite, were obtained for 2001-2005 at the 1km resolution. These variables included day and night land surface temperature (d/nLST), middle infrared reflectance (MIR), normalized difference vegetation index (NDVI) and enhanced vegetation index (EVI).

Regression models were built to investigate explanatory parameters for Culicoides variation, using a hierarchical approach. Models were produced for individual species, species groups, vector and non-vector groups, and all species combined. The small number of sampled sites precluded using calibration and evaluation datasets, so a leave-one-out regression allowed cross-validation to occur for C. obsoletus. Due to stability of coefficients and variance explained the process was not repeated for other species. Variograms of residuals were computed to identify the presence of second-order (local) effects. The Moran's I correlation coefficient was calculated for neighbourhood sizes of 1.5, 2 and 3km to identify residual spatial autocorrelation.

Between-farm variation in catches was up to 200% (403-66,000 midges), with 19 species trapped. The C. obsoletus group represented 62% of individuals trapped. There was high correlation in species abundance, with BT-vectors showing strong correlation with other vector species. Thirteen models were built, explaining between 73% (C. pulicaris) and 88% (C. fasciepennis) of catch variation. The C. obsoletus group model accounted for 81% and included number of sheep and water sources, alongside 4 NDVI, 2 MIR and 2 LST variables.

The best performing predictors were satellite-derived models, with 4 of the final models containing only satellite variables. In contrast to non-vector models, all BT-vector models included a host variable. The Moran's I statistic was insignificant at all neighbourhood sizes, except for C. festivipennis which exhibited negative spatial autocorrelation at the 1.5 and 2km scales.

Freely-accessible satellite data proved beneficial in modelling Culicoides distribution, enabling a strong C. obsoletus model to be built. Until now, no attempts were made to model the relationship between climate, host and environment on UK Culicoides distribution.





Diagnostics and intervention





ORAL: CONSTRUCTION AND CHARACTERIZATION OF THE INFECTIOUS CLONE OF THE HIGHLY VIRULENT WNV STRAIN IS-98-ST1

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Key words: West Nile virus, infectious clone, virulence, determinants

Introduction

West Nile virus (WNV) is a neurotropic flavivirus mainly transmitted through mosquito bites and whose reservoir hosts are wild birds. Equids and humans are incidental and dead end hosts and can develop severe neurological symptoms in 1-10% cases. WNV has a long history of circulation in Europe and the Mediterranean basin and has caused numerous but rather limited outbreaks in humans and equids since the late 1990's. One specific feature of WNV infections in Europe is the great diversity of WNV strains, belonging to at least 4 lineages. WNV epidemiology in Europe could well be changing with the huge spread of pathogenic lineage 2 infections (Hungary, Greece in 2010) and endemic circulation of WNV lineage 1a strains in new territories (Italy). One of the most important outbreaks in the Mediterranean basin, which took place in Israël in 1998, was associated to highly neuroinvasive WNV strain IS-98-ST1 (Lucas et al., Virology J., 1:9, 2004). To date, much remains to be explored about the neuroinvasiveness properties and the viral determinants of virus virulence of circulating WNV strains in Europe.

Objectives

We aimed at producing a new infectious clone, based on lineage 1a IS-98-ST1, for the characterization of the molecular determinants of European WNV strains virulence.

Results

A plasmid encoding the genomic RNA from WNV strain IS-98-ST1, flanked by a SP6 promoter for viral RNA in vitro transcription and HDV ribozyme for transcript termination at the end of the viral genome, was constructed by combining synthetic genes encoding the two extremities of viral genomic RNA and RT-PCR fragments. In vitro transcribed full-length viral RNA molecules were transfected into Vero cells and infectious virus particles were recovered after 3 days. The genomic RNAs from recovered WNV grown once on Vero cells and parental virus strain exhibited 100% identity. Comparative analysis of WNV derived from molecular clone with wild-type IS-98-ST1 strain showed no obvious differences in viral growth in vitro, antigenicity and neurovirulence in mouse model of WNV encephalitis.

Perspectives

A new molecular tool that is useful for the study of molecular determinants of WNV virulence has been generated. We take advantage of the high genetic stability of our one-piece infectious WNV cDNA clone to produce recombinant viruses through insertion of cassettes containing selected sequences from European WNV strains into the backbone of molecular IS-98-ST1. The pathogenicity of recombinant WNV will be assessed in vitro and in vivo. This project is part of the wider EuroWestNilenet project.

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ORAL: NOVEL REAL TIME RT-PCR ASSAYS FOR IDENTIFICATION AND TYPING OF EQUINE ENCEPHALOSIS VIRUS

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Key words: Equine encephalosis virus, EEV, Typing assays, real-time RT-PCR

The emergence and continuing spread of bluetongue virus (BTV) across the whole of Europe indicates that other orbiviruses may also represent a further threat to economically important livestock species and wildlife populations, both in Europe and other parts of the world. The recent identification of Equine encephalosis virus (EEV) in the Gambia and Israel, have highlighted a need for quick, sensitive and reliable molecular assays (e.g. by RT-PCR), to monitor the incidence and movements of these viruses. Based on recent experiences with BTV in Europe and elsewhere, these diagnostic tools could be used to rapidly identify infected animals and the serotype of EEV involved, helping to combat the spread of the virus and disease.

The EEV genome is composed of 10 linear segments of double-stranded RNA (dsRNA), each encoding at least one viral protein. The outer capsid proteins VP2 and VP5 that are situated on or near to the surface of the virus particle (encoded by genome segments 2 and 6) are more variable than components of the virus core, or the non-structural proteins. VP2 (encoded by Seg-2) is the outermost capsid protein (for BTV and closely related orbiviruses), and represents a primary target-antigen both for neutralising antibodies, and consequently for the development of type-specific, nucleic-acid-based diagnostic assays. The more conserved viral core-protein and/or non-structural protein genes can be used for the development of virus species-specific RT-PCR based diagnostic assays.

We report the development of real-time RT-PCR assays, for detection, identification and typing of any EEV strain/serotype, based on sequence data generated for Seg-1 Seg-9 and Seg-2 of each of the seven known serotypes (encoding the highly conserved viral polymerase 'VP1'; the conserved helicase 'VP6', and the most variable outer capsid protein 'VP2', respectively).

The ability of assays targeting Seg-1 and Seg-9 to detect any EEV strain, and the specificity of typing assays (targeting Seg-2), were evaluated using the seven EEV serotypes and isolates of closely related orbiviruses from the reference collection at IAH-P. These assays were shown to be reliable and specific. They did not cross-react with other closely related orbiviruses (group/species specific), or between EEV serotypes (type specific). Conventional primers were also designed for amplification and confirmation of RT-PCR results by sequencing.

These assays were used to identify EEV in six blood samples from Gambia (from 2009). Two of the samples were used to generate virus-isolates GAM2009/05 and GAM2009/06, which were typed as EEV-3 (Kaalplaas). Four further virus isolates from Israel (2008/2009) were also typed as EEV-3 (Kaalplaas). This demonstrates the potential of these assays for rapid screening of field samples. Further sequencing studies can be used help to identify the origins of individual virus strains.



ORAL: EPIZONE: BTV AND EHDV EPIDEMIOLOGICAL SITUATION

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Key words: BTV, EHDV, multiplex, molecular typing, diagnosis

Introduction

Bluetongue virus (BTV) and Epizootic haemorrhagic disease virus of deer (EHDV) are two species of the genus Orbivirus within the Reoviridae familly. Twenty-five serotypes of BTV and 8 serotypes of EHDV have been identified.

This paper describes the development of BTV and EHDV type–specific RT-PCR which have allowed to characterize new strains isolated from the Martinique (in the Caribbean Sea) and the La Réunion Island (in the Indian Ocean).

Materials and Methods

Study No 1:

In 2006, 30 cows (viro and seronegative for BTV) exported from France to the Martinique Island were sampled 10, 20 and 30 days post-importation.

The sera were tested by a competition ELISA (BT C-ELISA IDvet) and EDTA blood samples were used for PCR and viral isolation on embryonated eggs.

A sequence analysis of segments 2 (this segment 2 encodes VP2 which is the outer capsid protein involved in serotype specific serological reactivity) of the 24 serotypes of BTV (DNAStar Megalign) allowed to select a consensus pair of primers.

This pair of primers was used in PCR to amplify a part of segments 2 of isolated strains.

Study No 2:

In 2009, BTV-like clinical signs were reported in cattle of the French la Réunion Island. 121 animals with clinical signs were sampled. EDTA blood samples were used for BTV, EHDV-group-specific RT-PCR and viral isolation on embryonated eggs.

The segments 2 of the EHDV genome (encoding VP2) are clustered in 4 groups (A, B, C and D). We selected primers specific for each group. These pairs of primers were used in PCR to amplify a part of segments 2 of EHDV strains. The amplification products obtained with these primers were sequenced and compared (BLASTN) with those available on GenBank.

Results

Study No 1: Martinique

Thirty days post-importation, 56 % of animals seroconverted and 80 % were positive with a BTV group real-time RT-PCR.

The amplification products obtained with consensus primers from the RNA extract from isolated strains were sequenced and allowed to type the serotypes: 2, 9, 10, 17, 18, 22 and 24.

Sequence analysis of these strains showed that the origin of these viruses is unknown and that the serotypes 2, 10 and 17 do not originate from America.

Study N°2 : La Réunion Island

By BTV and EHDV-group-specific RT-PCR, 120 animals were detected as EHDV RT-PCR positive and 5 animals BTV and EHDV RT-PCR positive; moreover, one strain of BTV and 7 strains of EHDV were isolated in embryonated chicken eggs. The BTV virus was type as serotype 2.

The sequence of the amplification products (with the pair of primers specific of Group C) allowed to conclude that the EHDV serotype was 6. Sequence data showed that these strain was the cause of the EHD outbreak in the Island in 2003. This serotype 6 is also present in North Africa since 2006.



Discussion

The development of molecular diagnosis methods has greatly benefited from the availability of genomic sequences in international databases (GenBank,EMBL,...).

The selection of primers for PCR and their validation requires access to the nucleotide sequences of viruses of different serotypes and many strains of the same serotype. Since the emergence of the BTV serotype 8 in Europe, the laboratories have understood the need to share such genetic information for the benefit of the structures involved in the development of modern methods of diagnosis.



ORAL: BARCODING OF SWEDISH CULICOIDES FAUNA AS A TOOL FOR RAPID AND ACCURATE SPECIES DETERMINATION FOR VECTORS OF BLUETONGUE VIRUS

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 SVA^1

Key words: Culicoides, DNA Barcoding, molecular diagnostics

Certain species of biting midges (Diptera: Ceratopogonidae: Culicoides) are vectors for bluetongue virus (BTV). During the emergence of bluetongue virus serotype 8 (BTV-8) in northern Europe in 2006-2009 species of the obsoletus (C. obsoletus, C. scoticus, C. chiopterus and C. dewulfi) -and pulicaris (C. pulicaris, C.punctatus, C. impunctatus, C. lupicaris and C. newsteadi) complex have been implicated as main vectors. However, morphological identification of biting midges is difficult. Sample identification can be improved by using sequencing of a part of the mitochondrial gene Cytochrome oxidase c subunit I (COI) often called a barcode and assign this to morphological traits. Several studies have been made on COI with focus on the imicola- pulicaris - and obsoletus complex but the results have not been compared. Comparable sequences for other species of Culicoides is however lacking in the public databases. In this study we have sequenced 235 specimens from 37 species of Culicoides on COI and compared our material against available data in GenBank.

Culicoides were collected in Sweden from latitude 55-68°N, longitude 12-23°E. The head, wings and genitalia of individual biting midges were slide mounted and identified to species according to the keys of Campbell and Pelham-Clinton (1960) and Delécolle (1985). At least five specimens of each species were selected for the barcode study to get adequate sampling of intraspecific as well as interspecific variation. The remaining parts of the specimens were homogenized and a segment of the COI region were amplified with PCR and sequenced. A multiple alignment of all sequences were made and a NJ phylogenetic tree was constructed.

Our NJ tree generally was congruent with morphological based taxonomy. The sequenced individuals from each species in the obsoletus complex grouped closely together regardless of the geographic area from where they were collected, and formed a monophyletic clade. The species within pulicaris complex clustered as a monophyletic clade, and C. grisescens was non-monophyletic to this group as indicated by other studies. Culicoides newsteadi a species known to be variable grouped into three clusters within the pulicaris complex. One type grouped well together with sequences in Genbank, the other two was novel, and resembles morphologically C. halophilus. Culicoides lupicaris was also assigned but this type did not cluster together with sequences available in GenBank, though this species is known to have at least two morphotypes. Another 22 Culicoides species was sampled and showed high resolution at species level. However, for the specimens of C. festivipennis/C. clastrieri and C. salinarius/C. manchuriensis the sequences were shared among respectively species.

This study is a complement to the assembly of a reference library of barcode sequences for species of Culicoides in the paleartics. A complete library of barcodes may be used as rapid species determination of specimens in collections.



ORAL: SEQUENCING AND GENETIC CHARACTERIZATION OF ORBIVIRUSES AS A NEW APPROACH TO THEIR CLASSIFICATION, MOLECULAR IDENTIFICATION AND DIAGNOSIS

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 IAH^1

Key words: Orbivirus, Umatilla virus, Andasibe virus, Reoviridae

The genus Orbivirus is the largest genera within the family Reoviridae and contains 22 recognized virus species as well as 13 unclassified 'orbiviruses', some of which may represent still additional species. Bluetongue virus (BTV) is the type species of the genus. The orbiviruses are 'true' arboviruses that are transmitted by ticks and/or hematophagous insect vectors (phlebotomine flies, mosquitoes or Culicoides), with a wide host range that collectively includes domestic cattle, wild ruminants, equines, marsupials, sloths, bats, birds, humans, etc. The recent emergence of multiple BTV serotypes in Europe, multiple exotic BTV serotypes in the southern USA, as well as BTV-25, BTV-26 (see: Abstract by S. Maan et al) and other orbiviruses (Wallal, Warrego and Eubenangee viruses) in Australia (events that have been linked to climate change) illustrates a continuing and possibly increasing threat posed by these viruses.

Although sequence data are available for genome segments of multiple isolates of BTV, African horse sickness virus (AHSV), Epizootic haemorrhagic disease virus (EHDV), Equine encephalosis virus (EEV) and 5 other Orbivirus species, most genome segments of viruses belonging to the other 13 established Orbivirus species remain unsequenced. This makes molecular diagnosis (e.g. by RT-PCR assays) and unequivocal identification of individual virus strains (by phylogenetic analyses) more difficult.

We report full-genome sequence-data for Umatilla virus and Andasibe virus (an 'unassigned' isolate), as part of a project to generate representative data for the entire genomes of each Orbivirus species. Subsequent phylogenetic analyses have revealed the evolutionary relationships of these viruses and provide a molecular basis for classification of existing and novel isolates.

Comparisons of UMAV sequences to Stretch Lagoon orbivirus (SLOV) indicate that SLOV is a member of the Umatilla virus species and not a new species, as proposed earlier (Cowled et al., 2009). However, Andasibe virus is distinct from other orbiviruses, and a proposal will be made to ICTV to recognise it as a member of a distinct new Orbivirus species. Phylogenetic analysis of the T2 protein sequences (which determines the size and shape of the virus core), places this virus between EHDV and Eubenangee viruses suggesting that it may also have the potential to emerge when introduced in to susceptible populations.

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ORAL: AFRICAN SWINE FEVER VIRUS IN WILD BOAR – EXPERIMENTAL CHARACTERIZATION OF THE CAUCASIAN ISOLATE

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Key words: African swine fever virus, Caucasian isolate, wild boar

African Swine Fever (ASF) is one of the most important and complex diseases affecting pigs. The causative agent, African swine fever virus (ASFV), is a large, complex DNA virus of the genus Asfivirus within the Asfarviridae family. Vertebrate hosts are different members of the Suidae family. While warthogs can act as clinically inapparent reservoir, the virus causes severe multisystemic disease in domestic pigs. Little is known about the clinical course in wild boar. Due to its ability to replicate in soft ticks, ASFV is the only DNA virus hat can be classified as arthropod borne (ARBO).

While many countries of Sub-Saharan Africa are endemically infected, Europe was mainly affected by epidemic incidents. In 2007, ASF was reported in the Caucasus region where it concerned both domestic pigs and wild boar. So far, this outbreak could not be resolved. The presence of the disease in this region increases the risk to introduce the virus into the European Union (EU) and the involvement of wild boar raises concern. As seen with classical swine fever, the growing population of wild boar is most problematic for animal disease control, particularly if the infection gets endemic. Therefore, knowledge about disease dynamics is of utmost importance for risk assessment and strategy design, in particular since no vaccine is currently available against ASF.

For this reason, an animal experiment was carried out to define the symptoms in wild boar after oral infection with the Caucasian ASFV isolate. Moreover, transmission to co-housed domestic pigs was assessed. To mimic a natural infection route, six wild boar piglets (8 weeks of age, bred in captivity) were orally infected with 1 ml of a spleen suspension. The spleen originated from a domestic pig that had been infected with the Caucasian ASFV isolate in a previous experiment. The virus titre was 106.5 TCID50 per ml. Two days later, three domestic pigs of the same age were added. Body temperature and clinical signs were monitored daily. In addition, oropharyngeal and fecal swabs as well as blood samples were collected in regular intervals. Each animals was subjected to post-mortem examination.

All wild boar piglets developed an acute disease and died within 7 days. Apart from severe depression and reduced feed intake, only high fever was observed starting 3 to 4 days post infection (dpi). During post mortems, mainly enlarged and hemorrhagic gastrohepatic lymphnodes were observed. Two of the co-housed domestic pigs developed acute ASF 11 to 12 days post infection of the wild boar. These animals died one week later showing severe but unspecific symptoms. One domestic pig got infected later, developed fever 20 dpi, and was euthanized on day 25.

During the clinical phase, real-time polymerase chain reaction (PCR) was positive for all blood samples with first positive results 2 dpi. Oropharyngeal and fecal swabs were positive mainly on days 6 and 7 but showed sporadic detection also earlier in the trial.

Concluding, the Caucasian isolate was highly virulent for both wild boar and domestic pigs. Although all contact pigs died in the end, contagiosity was lower than it might have been expected. Based on these findings, it is not very likely that the Caucasian isolate has the potential to become endemic in European wild boar populations. In addition, rapid diagnosis can reliably be performed by real-time PCR.



ORAL: A NEW WAY OF PREVENTION RUMINANT'S DISEASES: CONSTRUCTION OF RECOMBINANT ATTENUATED GOATPOX VIRUS VACCINES

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Key words: Foot and mouth disease, peste des petits ruminants, capripox, recombinant attenuated goatpox vaccines, ruminants diseases

1. Introduction and Objectives

Foot and mouth disease(FMD), carpripox(CP) and peste des petits ruminants(PPR) are hazardous extremely serious disease against ruminants, and be classified as group A disease by OIE and group first disease in China.

Goatpox virus AV41 strains, an attenuated goatpox vaccine virus strains has been using in China against goatpox and sheeppox since 1984. In this study, we used the attenuated vaccine strain as live vector to express genes of FMDV and PPR. The construction of goatpoxvirus live vector will lay the foundation for the genetically engineered live vector vaccine of ruminant diseases.

2. Material and Methods

2.1 Goatpox virus AV41 strains, come from China Veterinary Culture Collection (CVCC). Type O and Asia1 strains of FMDV come from China National Food and Mouth Disease Reference Laboratory. A PPRV strain was identified from Xizang province of China.

2.2 Thymidine kinase (TK) gene of goatpox virus was selected as the extraneous gene cloning site. The TK gene and its flanking fragment was amplified with a pair of specific primers. The PCR product was 2078bp in length and the ORF of TK gene was 534bp encoding 178 amino acids. The identity analysis showed that AV41 shared 95.5%-100.0% identity rates with the reference strains in levels of nucleotides and amino acids. The results showed that TK gene was highly conservative.

2.3 To express foreign genes in recombinant virus with high efficacy, construction of a eukaryotic gene expression cassette P7.5-EGFP-P was obtained. FMDV P1-2A and 3C gene was amplified on FMDV genomic with designed primers. The purified target gene fragments were ligated into pGEM-T easy vector. Then the digestion product P1-2A3C gene was ligated into p-EGFP-N1-p7.5, yielding the whole reading frame named as p-EGFP-N1-p7.5-P12A3C, the structure of expression cassette was EGFP \leftarrow p7.5*20-p7.5*16 \rightarrow P12A3C. Then the segments was ligated into pUC119TK by the kpn I which was chosen to construction a framework of transfer vector Puc119-TK-EGFP-p7.5-P12A3C.

2.4 When transfecting BHK21 cells were infected by parent virus to make Lipofectamine TM2000 transfecting cell, at the 16th-48th after transfection, the transfected cells were observed under fluorescence microscope.

2.5 We constructed the transfer vector to express F and H gene of PPRV and P1-2A3C gene of FMDV type O and type Asia1 use the same methods as above.

3.Results

3.1 The transfer vector PTK-P7.5-EGFP-P transfected BHK-21 cells which infected GTPV AV 41. Specific fluorescence could be seen at 24h after transfection. Screened by Green fluorescent, RT-PCR and antigen capture ELISA. The results showed that P12A and 3C genes of FMDV and F and H gene of PPRV were expressed successfully. The tilter detection showed that the tilter of the recombinant was 105.5TCID50/0.1mL in BHK-21 cells. The results showed that P12A and 3C genes of FMDV and F and 3C genes of FMDV and H and F genes of PPRV were expressed successfully.

3.2 Animal experimental studies show that serum antibody against FMDV and CPV could be measured after vaccine sheep with the recombinant virus.



4. Discussion and Conclusions

This study is the first time to use an attenuated vaccine strain of goatpoxvirus of China as a vector to expressing FMDV and PPRV gene in the world. It advanced a new way of prevention ruminant's diseases, for example blue tongue, FMD, PPR, CP.

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Posters

BTV and other vector borne diseases

VBD 1: A LOW-COST EMERGENCE TRAP FOR THE CAPTURE OF NEWLY EMERGED FLYING INSECTS

THOMPSON, GEOFFREY¹; JESS, STEPHEN²; MURCHIE, ARCHIE²

Queen's University Belfast¹; Agri-Food and Bioscience Institute²

Key words: Emergence trap, Insect, Low cost

A low-cost trap for monitoring the emergence of flying insects was constructed out of a 5.5l plastic bucket. The traps were tested in the field to monitor Culicoides spp. and proved to be successful in capturing a wide range of flying insects. Additionally, the low cost, ease of construction, and suitability for storage of the traps, suggests that this trap design could be widely used by amateur or professional entomologists alike for scientific research. This poster outlines the trap design, its construction, and its durability in the field.

VBD 2: A NOVEL TRAPPING METHOD FOR ON-ANIMAL MONITORING OF ADULT CULICOIDES SPECIES

THOMPSON, GEOFFREY¹; JESS, STEPHEN²; MURCHIE, ARCHIE²

Queen's University Belfast¹; Agri-Food and Bioscience Institute²

Key words: Bluetongue, Culicoides, on-animal trap

An inexpensive, on-animal sticky trap was designed to monitor Culicoides spp. on agricultural livestock. The trap was constructed from 20cm x 10cm sheets of flexible plastic, coated in non-drying adhesive, and attached to the backs of cattle. Transparent and white traps were most successful in trapping Culicoides spp., capturing on average 15.5 and 17.1 midges respectively per trap in a 24-hr period. This poster describes the trap design, its construction, and compares effectiveness to traditionally used monitoring methods.

VBD 3: A RECENT VIRUS ISOLATE FROM KUWAIT WAS IDENTIFIED AS A NOVEL 26TH SEROTYPE OF BLUETONGUE VIRUS

<u>Maan, Sushila</u>¹; Maan, Narender S¹; Nomikou, Kyriaki¹; Batten, Carrie¹; Belaganahalli, Manjunatha N.¹; El Batel, Maha²; A.L. Oura, Chris¹; P.C. Mertens, Peter¹

IAH¹; VDL-ARC²

Key words: Bluetongue virus, BTV-26, Kuwait, sequence analysis

Members of the species Bluetongue virus (BTV) are the cause of bluetongue disease in ruminants. Serum taken from sheep and goats in Kuwait during 2010, were positive for BTV-specific antibodies when tested by cELISA at the VDL-ARC in Kuwait. The presence of BTV in blood samples was subsequently confirmed by the detection of viral RNA, using real-time RT–PCR assays at IAHP in the UK. The virus was successfully isolated in embryonated chicken eggs, from a sample of sheep blood from Kuwait, then grown in BHK cells (isolate KUW2010/02).

Sequencing of genome-segments 3 and 7 from KUW2010/02 (encoding orbivirus core-proteins VP3[T2] and VP7[T13]) and phylogenetic comparisons to other orbiviruses, showed highest nt / aa identity (76.6% / 88.9% and 81.2% / 97.7% respectively) to other BTV isolates, confirming its membership of this serogroup / virus species.

Nucleotide sequencing of Seg-2/VP2 from KUW2010/02, and comparisons to BTV serotypes 1 to 25, showed nt/aa identity levels of less than 63.9% / 61.5%, consistent with its identification as a novel 26th BTV type. Antisera against BTV-1 to BTV-25 also failed to neutralise KUW2010/02, supporting this conclusion. When inoculated into sheep, this BTV isolate caused only mild clinical signs.

VBD 4: BLUETONGUE MONITORING AND SURVEILLANCE IN EUROPE AND THE POTENTIAL ADDITIONAL RISK POSED BY SEROTYPE 8 – A SYSTEMATIC LITERATURE REVIEW

DHOLLANDER, SOFIE¹; GERYELEMEYER, A.¹; GERVELMEYER, ANDREA¹

European Food Safety Authority¹

Key words: bluetongue, epidemiology, monitoring, surveillance, design prevalence, transmission

In October 2010, the European Commission asked the European Food Safety Authority (EFSA) to provide a scientific opinion on the possible additional risk posed by bluetongue serotype 8 compared to other serotypes and to assess the potential effect of this on bluetongue epidemiology. In addition, EFSA was asked to recommend on epidemiological parameters such as the expected prevalence under different circumstances and the size of a geographical relevant area for bluetongue monitoring and surveillance programmes.

Two working groups, consisting out of 8 independent experts and 3 members of the EFSA Animal Health and Welfare Panel were created to answer the terms of reference. A systematic literature review was considered as the most appropriate approach in the given time-frame, due to its methodological rigor and its transparent nature. A review protocol was developed, including 3 review questions on which the search equations to search 3 electronic databases (CAB Abstracts, WoS, PubMed) were based. Two reviewers independently screened the 1396 automatically retrieved papers for their relevance, based on criteria that were agreed upon by the working groups. The 247 papers that were considered relevant were further appraised by the working group members using a-priori set eligibility criteria, after which the evidence was extracted from the eligible papers. The review process was carried out in DistillerSR and documented adequately to allow readers to critically appraise the criteria used in selection of papers, the interpretation of the results and, if necessary, to repeat or update the review. A summary of the retrieved evidence is presented and discussed.
VBD 5: BLUETONGUE: A NORTHERN IRELAND PERSPECTIVE

<u>JESS, STEPHEN¹</u>; MURCHIE, ARCHIE¹

Agri-Food and Biosciences Institute¹

Key words: Culicoides, bluetongue, surveillance, transplacental infection

Agriculture in Northern Ireland is reliant on its livestock sector which includes 1.6M cattle and 1.9 M sheep with a current value estimated at £M737 (Anon 2009). In response to the introduction of BTV-8 into northern Europe, an entomological surveillance programme was initiated in Northern Ireland to determine the geographic and seasonal distribution of biting midges (Culicoides spp.) that may potentially be involved in transmission of bluetongue viral disease.

Since October 2007, a total of 19 Onderstepoort-type suction light traps at 14 locations across Northern Ireland has examined the geographic and seasonal distribution of adult Culicoides spp.. This complemented a similar study in southern Ireland comprising 34 farm sites, which provides a comprehensive database that will in future be used to develop mathematical models for analyses of Culicoides population dynamics and behaviour.

Representative species from Obsoletus and Pulicaris complexes were found at all sites, including C. chiopterus, C. dewulfi, C. obsoletus, C. scoticus and C. pulicarus. Adult midge activity declined throughout the latter part of the year and a bluetongue vector-free period was declared in late December 2007. During this period, and with no evidence of iatrogenic infection, a bluetongue outbreak occurred in Northern Ireland. A number of pregnant cattle were imported to a farm in Northern Ireland from the Netherlands. Post-importation tests indicated that some of the pregnant cattle had antibodies to the bluetongue virus but were not viraemic (RT-PCR negative). Two of the cattle subsequently gave birth to three calves that indicated bluetongue virus infection (RT-PCR positive) with one calf demonstrating viraemia. This was the first field evidence transplacental transmission of bluetongue virus. Two further viraemic animals (one newly calved heifer and one milking cow) were disclosed and circumstantial evidence suggests contact spread of the bluetongue virus with oral transmission being the most probable route of infection.

VBD 6: CHEMICAL COMPOSITION OF SILAGE RESIDUES SUSTAINING THE LARVAL DEVELOPMENT OF THE C. OBSOLETUS/SCOTICUS COMPLEX SPECIES (DIPTERA: CERATOPOGONIDAE)

<u>ZIMMER, JEAN-YVES</u>¹; SAEGERMAN, CLAUDE²; LOSSON, BERTRAND²; BECKERS, YVES¹; HAUBRUGE, ERIC¹

Gembloux Agro-Bio Tech (University of Liege)¹; University of Liege²

Key words: Culicoides, Bluetongue vectors, chemical requirements, silage residues, C. obsoletus/scoticus complex

Culicoides (Diptera: Ceratopogonidae) are biological vectors of bluetongue virus (BTV). Bluetongue is a viral disease that affects domestic and wild ruminants. Since its recent emergence in northern Europe, this disease has caused considerable economic losses to the sheep and cattle industry. The biotopes and more particularly their chemical characteristics which are suitable for larval development of the main vector species are still relatively unknown. This study shows that the larvae of biting midges belonging to the Culicoides obsoletus and Culicoides scoticus species are able to breed in different types of silage residues (maize, grass, sugar beet pulp and their combinations). The chemical composition of substrates strongly influences the presence of immature stages of these biting midges. Thus, the lignin seems to favor their presence; it could play the role of a physical support for the semi-aquatic larvae. In contrast, increasing concentrations of calcium and potassium are negatively correlated with the presence of these two species. These data will help to locate and monitor the breeding sites of these species and could contribute to the control of these insects in the farms.



VBD 7: DEVELOPMENT OF AN ANIMAL MODEL FOR THE BLUETONGUE VIRUS SEROTYPE 8

<u>STOCKHOFE-ZURWIEDEN, NORBERT</u>¹; VAN GENNIP, RENÉ G.P.¹; BOONSTRA, JAN¹; BACKX, ANOEK²; WRIGHT, ISABEL M.³; POTGIETER, CHRISTIAAN A.³; WIERINGA-JELSMA, TINKA¹; MARIS-VELDHUIS, MIEKE A.¹; SWANENBURG, MANON¹; VAN RIJN, PIET A.¹

CVI¹; Institut de Veille Sanitaire²; Deltamune³

Key words: Bluetongue serotype-8 animal-model vaccine

In 2006, bluetongue virus serotype 8 BTV8\net06 (IAH collection nr. BTV-8 NET2006/04, Maan et al., 2008) was first detected in the Limburg province, the southern panhandle, of the Netherlands bordering Germany and Belgium. BTV8 has invaded Belgium, Netherlands, Germany, Luxemburg and the northern France. After overwintering, BTV8 reoccurred in several countries in 2007 and following years resulting in the largest BT-outbreak ever recorded. In many aspects, this outbreak strain is different from other BTV-strains, like spread by N-W European species of Culicoides, transplacental and oral transmission, and cause of severe disease in cattle (Backx et al., 2007, 2009, Meiswinkel 2007, Dijkstra et al., 2008). The BTV serogroup, family Reoviridae, genus Orbivirus, contains at least 24 serotypes defined as inducing no or very low levels of cross neutralization. Bluetongue (BT) is an arthropod-borne disease; transmission to ruminants, including cattle, sheep, and goats, occurs by bites of species of Culicoides. Since 1998, BTV serotypes 1, 2, 4, 9, and 16 have invaded European countries around the Mediterranean Basin, but these BTV serotypes have not been spread northwards so far.

We have performed research on the basis of BTV8\net07 (IAH collection nr. BTV-8 NET2007/01), the 1st reported BTV-infection in 2007. Extensive sequencing studies were performed on virus passaged once on embryonated eggs, the 3rd passage on BHK21 or KC cells, and directly from blood. All ten complete genome segments were reversely transcribed, PCR-amplified (Potgieter et al., 2009), and sequenced with 454 Roche GS FLX technology. No differences were found after one passage in eggs and three passages in BHK21, and only two differences were found after passage in KC cells. This demonstrated that BTV8\net07 is genetically stable in BHK21 cells, and thus can be reproducibly produced for challenge experiments.

In parallel, we have performed several animal trials with BTV8\net07 in order to develop a reproducible animal model for vaccination/challenge experiments. For this purpose, an infection experiment was performed in sheep and cattle with vireamic blood, and with the above described passages. The dose/animal was normalized by quantitative PCR-signals, therewith realizing that the infective dose/animal could be different between these inocula, in particular with respect to the vireamic blood.

From the summarizing results, we concluded that the 3rd passage in BHK21 was suitable as challenge inoculum, and that sheep show the most obvious clinical signs. In following experiments, this 3rd passage in BHK21 was further investigated in dose-response trials. In addition, virus titers were determined to define the dose of the optimized challenge inoculum. Details and results of these animal trials will be presented and discussed. In summary, a satisfactory and reproducible sheep model is developed in order to test and compare the efficacy and safety of vaccine (candidates) for Bluetongue.

- Backx et., 2007 Vet. Record 161, 591-593.
- Backx et al., 2009 Vet. Microbiol. 138, 235-243.
- Meiswinkel et al., 2007 Vet. Record, 161, 564-565.
- Dijkstra et al., 2008 Vet. Record. Letter 422.
- Potgieter et al., 2009. J.Gen.Virol. 90, 1423-1432.

VBD 8: DEVELOPMENT OF GENETICALLY DIFFERENTIATING VACCINE FROM WILD TYPE BLUETONGUE VIRUS (GENETIC DIVA)

VAN RIJN, PIET A.¹; VAN DE WATER, SANDRA G.P.¹; VELDMAN, DANIEL¹; VAN GENNIP, RENÉ G.P.¹

CVI^1

Key words: Bluetongue diagnostics genetic DIVA

Bluetongue virus (BTV), family Reoviridae, genus Orbivirus, contains ten double stranded RNA segments encoding at least ten viral proteins. Bluetongue (BT) is an arthropod-borne disease; transmission to ruminants, including cattle, sheep, and goats, occurs by bites of species of Culicoides. Vaccination against several serotypes of bluetongue virus (BTV) with inactivated vaccines and/or modified-live vaccines has been carried out for years all over the world, including in the European countries affected by BTV serotype 8. Serological monitoring for BTV-infection is not possible with commercially available ELISAs in these vaccinated populations. These ELISAs detect seroconversion irrespective to (the serotype of) the used BT-vaccine, and the field strains of BTV. Alternatively, sentinel programs are in use to detect BTV-circulation by seroconversion in depicted (nonvaccinated) sentinel animals in a vaccinated ruminant population. However the use of inactivated vaccines also opens the way to detect BTV-circulation by PCR-diagnostics in, or shortly after, the BT-season. Natural BTV-infection can be detected in cattle for about 100-200 days post infection, whereas inactivated vaccines usually do not result in PCR-positivity. However, a few reports have shown PCR-positivity after vaccination with inactivated vaccines.

In the national reference institute for Bluetongue in the Netherlands, high throughput real time PCR-diagnostics, based on genome segment S10, has been routinely used since August 2006 (Backx et al., 2009). Since then, more than 200,000 samples were tested. This PCR-assay reliably detects with high diagnostic sensitivity and specificity all members of the BTV serogroup irrespectively of serotype and origin.

The aim of this study was the development of a genetically modified vaccine, which will not be detected by this PCR-assay resulting in genetic DIVA* (*differentiating vaccinated from infected animals). Recently, we have developed genetic modification systems for BTV (van Gennip et al. 2010, 2011), and here we have used this technology to genetically modify the target of the PCR-assy. For this purpose, a mutated S10 genome segment was generated containing mutations in the location of the reverse primer. All introduced changes were silent mutations with respect to the translated amino acid sequence of NS3/NS3a. Indeed, mutant BTV could be rescued and was not detected by the PCR-assay. Incorporation of this mutated S10 genome segment in future vaccine candidates will avoid false positive detection after vaccination with inactivated vaccine. Furthermore, by use of modified-live vaccines this genetically modified S10 will not be detected by our PCR-assay. However, the risk on reassortment events between modified-live vaccine and wild type virus must be assessed and evaluated with regard to the diagnostic sensitivity of this PCR-assay.

- Backx et al., 2009. Vet. Microbiol. 138, 235-243.
- van Gennip et al., 2010. BMC Virology Journal 7, 261-267.
- van Gennip et al., 2011. in preparation and poster/presentation.

VBD 9: DEVELOPMENT OF REAL-TIME-RT-PCR ASSAYS FOR THE DETECTION EPIZOOTIC HAEMORRHAGIC DISEASE VIRUS

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Key words: Epizootic Haemorrhagic Disease Virus, Reverse Transcription, Real-Time PCR

Epizootic hemorrhagic disease virus (EHDV) is a member of the genus Orbivirus, family Reoviridae, and is closely related to bluetongue virus (BTV). EHDV infects wild ruminants, causing a frequently fatal haemorrhagic disease. However, it can also cause bluetongue-like disease in cattle, involving significant levels of morbidity and mortality, highlighting a need for more rapid and reliable diagnostic assays. EHDV often causes death in white-tailed deer and, less frequently, a bluetongue-like illness in cattle. The association of EHDV with clinical hemorrhagic disease in sheep and cattle is rare, but the infection is typically asymptomatic. EHDV has a genome composed of 10 dsRNA segments. A Real-Time-RT-PCR was developed and evaluated for detection of EHDV in cell culture and clinical samples.

Selection of primers and an oligonucleotide probe was carried out using the program Oligo 4.0 on the basis of the nucleotide sequence in GenBank [NCBI GI: AM745001; AM744981]. The Real-Time-RT-PCR was performed on the RotorGene-6000 cycler («Corbett Research», Australia), and the data obtained were analyzed using the software provided with the machine. The specificity of this method was confirmed by sequencing the PCR amplicons. Oligonucleotide primers were developed targeting NS1 gene, for use in conventional Real-Time-RT-PCR assays to detect reference strains Alberta and New Jersey.

VBD 10: DISTRIBUTION OF CULICOIDES SPECIES IN POLAND IN 2009

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NVRI¹; Maria Curie-Skłodowska University²

Key words: Culicoides, bluetongue, midges, light-traps, vectors

Introduction and Objectives

Since August 2006, Northern European countries have been affected by large epidemic of bluetongue. BT is an infectious, non contagious disease transmitted by biting midges belonging to Culicoides species. The occurrence of bluetongue in new geographic area where is no evidence of C. imicola, suggest that other Culicoides species play role as a virus vector. Recently, other than Culicoides routes of BTV transmission is discussed. The aim of the study is to estimate a total number, taxonomy, activity period and geographical distribution of Culicoides species in Poland, in 2009.

Material and Methods

Light traps (Ondestepoort type) were located in 22 farms all over Poland territory, nearby susceptible animal housings. Collection was made from the beginning of April to the half of December. Light traps were operated from one hour before sunset to one hour after sunrise, once a week. Trapped insects were collected in labeled jars with 70% ethanol, and were send to laboratory. Culicoides species were separated from other insects, and then classified by microscopic examination.

Results

A total of 778 collections were made. Range between trap locations in number of catches varying from 29 to 50. Over one milion insects was collected, 395 434 of them belonged to Culicoides complex, and 368 823 migdes, belonging to 6 species, capable to transmit bluetongue virus: C. obsoletus, C. scoticus, C. chiopterus, C. dewulfi, C. pulicaris and C. punctatus. Also it was found out 9 other species of Culicoides, placed in annex to EU regulation 1266/2007. 75% among all trapped Culicoides species comprised C. obsoletus. 14% comprised C. punctatus. 3% comprised C. pulicaris. Only 11 insect belonging to C. dewulfi was found, trapped in 3 locations.

Discussion and Conclusions

The number of Culicoides trapped in Poland was related to the geographical location of the trap. High density was shown in Dolnoslaskie, Wielkopolska, Malopolska, Podkarpackie and Lubelskie regions, south-western Poland. 15 Culicoides species were found during catching season, most of them belonged to C. obsoletus and C. punctatus. Presence of blood fed female (parous and blood fed) on the beginning of April has demonstated that Culicoides were active even earlier than thought before or even that Culicoides can process life cycle during the winter within animal housings. Entomological research was continued in 2010 to obtain more precise data.

VBD 11: DISTRIBUTION OF MAIN BLUETONGUE VIRUS VECTORS ON BELGIUM FARMS

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Key words: Culicoides, Bluetongue, Monitoring, UV light traps, Cattle and Sheep

Bluetongue (BT) is a non-contagious vectorborne disease of domestic and wild ruminants. Since its emergence in northern Europe in August 2006, this viral epizooty has caused considerable economic losses to the sheep and cattle. In 2007 and 2008, BT virus (BTV) serotype 8 continued its spread across Europe. The biological vectors of BTV are biting midges of the genus Culicoides. This genus counts about 1.400 species, but only some of them are involved in the transmission of this virus.

During this study undertaken in 2007, populations of biting midges were monitored on a cattle (50°33' N, 4°41' E) and a sheep farm (50°26' N, 5°01' E) in the province of Namur (Belgium). UV light traps were placed both indoors (cowshed and sheepfold) and in nearby meadows. Results of trappings showed that Culicoides are most abundant close to livestock buildings than in surrounding meadows, according to the factors 16 (sheep farm) and 22 (cattle farm); meadows had however a greater species diversity and therefore a lower percentage of species known as vectors of BTV. The two species of the C. obsoletus/scoticus complex predominated for all trappings, particularly in the sheepfold and in the cowshed with 98% and 82% respectively. Females caught by light trappings were much more numerous than males; females represented indeed over 99% of individuals from the sheepfold and the cowshed. In the meadows, males however accounted for about 15% of Culicoides biting midges trapped. The statement of minimum and maximum temperatures, as well as observation of females' physiology completed this study. Percentage of nulliparous females is higher indoors (49% and 65% for sheepfold and cowshed respectively) than in nearby meadows (29% and 17% respectively). This observation could be explained by the presence of breeding sites inside livestock buildings, which would participate to the persistence of BTV from year to year despite fairly harshwinters.

VBD 12: EPIZONE: DISSECTION OF THE HUMORAL IMMUNE RESPONSE TO BLUETONGUE VIRUS INFECTION

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 FLI^1

Key words: BTV, Immune response, Immunoglobulin isotypes, ELISA

Competitive ELISAs for the detection of Bluetongue virus (BTV) antibodies are well established and widely available. Recently, double antigen (sandwich) ELISAs have been introduced that can detect the antibody response to a BTV infection earlier than competitive tests, and are highly sensitive for vaccine-induced antibodies.

On the other hand, previous experiments have demonstrated a substantial decrease in detected antibody levels in the double antigen ELISAs after a peak early in infection, which might have an impact on diagnostic sensitivity. The observed decrease is attributed to immunoglobulin isotype switching in combination with a reduced sensitivity of the sandwich tests to IgG. No data on the kinetics of individual antibody isotypes after BTV infection are available, however, and the isotype-specific sensitivity of the commercial assays has not been evaluated.

In this study, eight cattle and eight sheep were infected with German isolates of BTV serotypes 6 and 8 (four animals of either species per serotype). During the first two weeks after infection, blood samples were taken daily; followed by three samples per week for the next six weeks. Successful infection was confirmed by real-time RT-PCR and virus isolation.

Serum samples are analysed by commercially available competitive and double antigen ELISAs and serum neutralization tests. The post-infection kinetics of BTV-specific bovine IgM, IgG and IgA are determined by custom ELISAs. Correlations between the signal strength in the commercial assays and the isotype composition of samples will be further evaluated by isotype-specific immunoglobulin depletion.

Results and conclusions will be presented at the conference. This study will provide valuable insights into the humoral immune response to BTV infection, with a focus on the implications for BTV diagnostics.

VBD 13: EPIZONE: RELATIVE VIRULENCE OF EUROPEAN BLUETONGUE VIRUS SEROTYPES 6 AND 8

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 FLI^1

Key words: BTV, Immune response, Innate immunity, Interferon

After its introduction to Central Europe in 2006, Bluetongue virus (BTV) serotype 8 (BTV-8) has spread widely. When BTV serotype 6 (BTV-6) was introduced to the same area two years later, it did not spread to any meaningful extent. There is little or no cross-protective immunity between serotypes, and the failure of BTV-6 has been attributed to its close phylogenetic relation to an attenuated vaccine strain. The mechanism of attenuation, however, is unknown.

In a preliminary study, the relative virulence of German isolates of BTV-6 and BTV-8 was investigated by experimental infection of sheep and type I interferon receptor deficient IFNAR-/- mice. While both serotypes were found to be highly virulent in the mice (with an LD50 of about 10^-1 TCID50 for both), the early replication of BTV-6 in sheep was significantly reduced compared to BTV-8 (over 120-fold on day 3 and 7-fold on day 5). The divergent observations in IFNAR-/- mice and sheep suggest that the attenuation of European BTV-6 is related to the interferon system.

Based on this preliminary data, an extended experiment was designed. Eight cattle and eight sheep were infected with BTV-6 and BTV-8 (four animals of either species per serotype). During the first two weeks after infection, blood samples were taken daily; followed by three samples per week for the next six weeks. Serum was separated immediately and stored at -80 °C until analysis. Peripheral blood mononuclear cells were purified using Ficoll-Paque[™] PLUS, suspended in TRIzol® Reagent and stored at -80 °C as well.

Using real-time RT-PCR, differences in virus replication between the serotypes will be examined. Biologically active type I interferon in serum samples will be quantified with an Mx/CAT reporter gene assay (Fray et al., 2001, J Immunol Methods 249[1-2]:235-44), and cytokine expression in PBMC will be analysed by real-time RT-PCR (Zhang et al., 2006, J Comp Pathol 134[1]:56-62).

Results and conclusions will be presented at the conference. If a difference in interferon induction between BTV-6 and BTV-8 can be confirmed, this has important implications for BTV vaccine design by reverse genetics.

VBD 14: ESTIMATING THE WITHIN-HERD REPRODUCTION RATIO (R0) OF BLUETONGUE SEROTYPE 8 IN 2007 IN THE NETHERLANDS

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Key words: BTV-8; transmission; cattle; field data

Bluetongue virus serotype 8 (BTV-8), emerged in North-West Europe in 2006. In 2007, one of the affected countries (the Netherlands) implemented a sentinel network of dairy cattle. Seronegative cattle within sentinel herds were selected and entered a monthly sampling program, from July until December 2007. This data offered the opportunity for estimating transmission parameters for BTV-8 based on serological field data, which is important for quantifying the risk of BTV-8 in an epidemic situation. With this knowledge decisions concerning monitoring, eradication or vaccination programs can be made in countries in which BTV-8 emerges for the first time. Thus, the goal of this study was to quantify the transmission of BTV-8 (R0) within a herd based on the 2007 sentinel data.

R0 was calculated using, a vector-borne transmission model. In this model, a Culicoides could become infected by biting a BTV-8 infectious cow and transmission taking place. An infected Culicoides became infectious when it survived the extrinsic incubation period and could then infect susceptible cows by biting and transmission taken place. Cows were on average infectious for 25 days before they recovered, Culicoides stayed infectious throughout their lives. In the default model, transmission parameters like the proportion of infectious cows, infectious Culicoides and vector over host density were calculated from field data. Furthermore, literature and temperature based assumptions were made for recovery rate, Culicoides biting rate, extrinsic incubation period, Culicoides mortality rate, the probability of BTV-8 transmission from vector to host and the probability of BTV-8 transmission from host to vector.

R0 could be estimated for 419 time intervals. The mean R0 was 3.8 and the median R0 was 2.9 (5th percentile=2.2; 95th percentile=9.0). Median R0 values differed between regions and months. Values of R0 decreased in months in which conditions for Culicoides survival and transmission declined due to decreasing temperatures. In addition, the median R0 value for BTV-8 in the northern region was significantly lower compared to the southern region, while the natural habitat of the Culicoides and the average daily temperatures do not differ between the regions in the Netherlands. Possibly, the lower R0 values in the northern region are associated to the slower start of the epidemic in the north. From September-October on, BTV-8 transmission within-herds in the northern region increased. However, from that moment on, conditions for Culicoides became less favorable because of the decreasing temperatures and thus the R0 remained low.

The median vector to host ratio was calculated from our field data at 159 (5th percentile 80; 95th percentile=2132). The vector to host ratio differed between months, with the highest number of Culicoides per cow between October and November. In this period, the conditions for Culicoides became less optimal because of the declining temperatures and it was expected that BTV-8 transmission between cattle stopped. Nevertheless, the cattle field data showed that in some cases, there still was transmission of the virus in the cattle population. This study gives within-herd estimations for R0 for the BTV-8 epidemic, based on serological field data. This R0 seems to represent the spread of BTV-8 and these transmission rates may apply to countries in which BTV-8 emerge, given a similar climate, grazing patterns and barn type as North-West Europe.

VBD 15: Evidence of bluetongue virus serotypes 1 and 16 in oryx antelope imported from the Sultanate of Oman during the quarantine period on Veliki Brijun Island, Croatia

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Key words: oryx antelope, quarantine, bluetongue virus serotype 1, bluetongue virus serotype 16, Croatia

On 4 March 2010, ten individual oryx antelopes were imported into Croatia from the Sultanate of Oman. The oryx antelopes were placed into quarantine on the island of Veliki Brijun, situated 3 kilometres offshore of the southwestern part of the Istrian Peninsula. The animals were intended to spend six months on the island before being transported to their final destination in the United Kingdom. The test results accompanying the animals indicated a negative test for bluetongue (BT). During guarantine, the animals were serologically tested for the BT virus, using cELISA. Four oryx tested positive for BT virus antibodies. Blood samples of the oryx were sent for testing to the Reference Laboratory for bluetongue for the EU and the World Organisation for Animal Health (OIE) in Pirbright. The BTV serotype 16 was identified in one animal and BTV serotype 1 in two animals, while the PCR method did not determine the type of BTV in the fourth animal. In order to establish the potential spread of the BT virus vector, the competent body began an entomological survey of Culicoides biting midges (Diptera: Ceratopogonidae), using Onderspoort blacklight suction traps at two locations on Veliki Brijun island (in the oryx quarantine and in the sheep stable near to the quarantine site). Insect samples were collected every second day until 5 April, when the animals were transported back to the Sultanate of Oman. The insect samples were analysed by the Croatian Veterinary Institute in Zagreb. A total of six catches were captured in the oryx quarantine facility, however, none of the samples included insects of the genus Culicoides. One of the six catches collected near to the sheep stables was identified as a vector of the Obsoletus Complex. Based on the measures taken and the survey results obtained, it is very important that no vectors of the BT virus were identified in the oryx quarantine.

VBD 16: GENETIC STUDY OF BLUETONGUE VIRUS STRAIN "TAPKHAR"

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Key words: bluetongue, sequence analysis, origin

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 virus caused severe disease in sheep-farms. The morbidity rate of adult sheep was 58,3 %, the mortality rate was 66,3 %. The virus was isolated from blood samples taken during this outbreak and replicated in PSGK. According to the neutralization test, the agent was shown to belong to serotype 16. It was characterized by biological characteristics and further deposited as strain "Tapkhar" in NRIVVaM strains collection. The disease was brought under control in a short time.

Until this investigation the virus was not genotyped by molecular genetic methods. In our investigation, the sequence analysis of three virus segments (seg-2, seg-7, seg-10) was performed. Subsequent BLAST analysis of seg-2 indicated that the strain belongs to an eastern group of viruses and is closely related to the SAD2004/04 BTV-16 (100% identity), the outbreak in Sardinia during 2004 was also caused by BTV-16, although the strain involved proved to be identical to the BTV-16 vaccine that was used in Italy during 2004 (Savini et. al., 2008). The Italian field virus has been shown to be a reassortant containing Seg-2 derived from the BTV-16 vaccine but with Seg-5 derived from the BTV-2 vaccine strain that was also used as part of the multivalent vaccine in Israel (Batten et al., 2008). These investigations are showing the analogous direction of origin the Russian strain "Tapkhar".

VBD 17: IDSCREEN® BLUETONGUE DIVA COMPETITION: NEW TOOL FOR NEWS CHALLENGES

MOCKEY, MICHAEL¹; LECOQ, ANNA¹; COMTET, LOIC¹; POURQUIER, PHILIPPE¹

IDVET¹

Key words: Bluetongue, ELISA, DIVA

The mass vaccination campaigns in Europe appear to have been successful in controlling the spread of BT disease. Recent reports confirm the complete absence of BTV-8 from Western and North Europe during 2010. Unfortunately, several outbreaks of other serotypes have been reported (BTV-1, BTV-2 and BTV-4) around the Mediterranean Basin and widespread vaccination campaign leads to large-scale VP7 seroconversion. In this context, it becomes impossible to distinguish vaccinated from naturally-infected animals (DIVA) using the currently available immunoassays. To respond to the evolution of BT serodiagnostics in Europe and using original properties of the non structural protein 1 (NS1), IDVET has developed an innovative competitive ELISA test, IDScreen® Bluetongue DIVA Competition, which demonstrates its ability to differentiate naturally-infected animals from naïves or vaccinated animals using commercially available inactivated vaccines. Results will be presented and discussed.

VBD 18: IDSCREEN® BLUETONGUE VIRUS SEROTYPE 8 COMPETITION

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 $IDVET^1$

Key words: Bluetongue, BTV-8, ELISA

In 2006, BTV serotype 8 (BTV-8) outbreaks occurred almost simultaneously in Belgium, France, Germany, and the Netherlands, leading to the greatest epizootic disease on record in naive sheep and bovine herds. To limit the significant damage caused to breeders by the BTV and to facilitate safe trade in live animals, widespread vaccination programs have been implemented using inactivated vaccines. This vaccination campaign succeeded and no cases of BTV-8 in Western and Northern Europe were reported in 2010. However, livestock turn-over associated with the end of systematic vaccination against BTV might lead to recirculation of this serotype, outbreaks of BTV-1, BTV-2 and BTV-4 occurred recently in Italy, Spain, Morocco and Algeria. In this context, IDVET has developed an innovative competitive ELISA test. Based on the VP2 structural protein of BTV, IDScreen® Bluetongue Serotype 8 Competition is a serotype-specific test which allows for the differentiation of BTV-8 infection from at least 6 other serotypes (BTV-1, -2, -4, -9, -10 and -16). Preliminary validation results will be presented and discussed.

VBD 19: INFECTION OF BRITISH CATTLE WITH 2 DIFFERENT STRAINS OF EHDV-6

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IAH¹; Laboratoire Service International²

Key words: EHDV, ELISA, PCR, Pathogenicity

Epizootic Haemorrhagic disease (EHD) has recently been classified as a notifiable disease. In recent years outbreaks of EHD have occurred on the outskirts of Europe in Morocco (2006), Israel (2006) and Turkey (2007). Clinical signs are similar to those observed with Bluetongue (BT) including conjunctivitis, oedema, lameness and decreased milk yield, therefore differential diagnosis is required. An initial suspicion of EHD, based on observation of clinical signs, can be confirmed by virus isolation and characterization, nucleic acid identification, or serological testing.

In this study two groups of five Holstein-Friesian cattle were infected with two different strains of EHDV (MOR2006/17 KC3 EHDV-6 and TUR2007/01 KC2 EHDV-6) to assess the pathogenicity of these viruses in UK cattle. Clinical signs were monitored throughout the duration of the experiment. Levels of both EHDV RNA and antibodies were measured at various timepoints throughout the experiment using EHDV specific PCRs and antibody-detection ELISAs.

A commercially available EHDV real time RT-PCR (Kit TaqVet Epizootic Hemorrhagic Disease Virus, Laboratoire Service International, France) was compared to other available real-time. Validation data assessing the sensitivity of these assays will be presented. In addition the sensitivity of available antibody detection EHDV ELISA tests (Laboratoire Service International, France) will be assessed and the serological response of cattle to EHDV-6 will be measured and compared.

VBD 20: INTERNATIONAL NETWORK OF REOVIRUSES, BLUETONGUE AND AFRICAN HORSE SICKNESS, IN THE MEDITERRANEAN BASIN AND EUROPE: MED_REO_NET

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Key words: Bluetongue, African horse sickness, Europe

The work plan of MED_REO_NET foresees a number of interrelated tasks, with measurable deliverables and milestones. Six work packages (WP), divided in vertical activities dealing with either tools or data needed for surveillance and horizontal or transversal activities dealing with more integrative activities, have been identified.

The vertical activities are the regional surveillance of virus activity and vaccination (WP1), the regional surveillance of vectors (WP2), molecular epidemiology (WP3), and the horizontal activities are Databases, web design and GIS (WP4), risk assessment (WP5), and meetings and dissemination (WP6).

Specifically the plan aims at:

1. Strengthening and harmonizing of the surveillance of BTV/AHSV/EHDV infections in Europe by the evaluation of protocols, and the harmonization of available tools for surveys, viral identification and vaccination strategies.

Strengthening the Culicoides surveillance in Europe and neighboring countries with specific objectives of evaluation and harmonization of surveillance protocols, collection methods, vector identification (morphology and molecular biology) and modeling of vector distribution.
Identification of the distribution, origins and movement of different BTV and EHDV strains, by characterization of well documented isolates of different serotypes.

4. Developing a Culicoides-borne virus network, through a website which will provide a platform of internet-based tools to be used by partners and other interested parties, to enable i) Effective information exchange between partners, ii) To develop a web-enabled Geographic Information System (GIS) to present epidemiological data and to allow a rapid spread of information related to the diseases, iii) to provide a real-time interactive mapping system of the main epidemiological aspects to facilitate decision-making process and management of control activities at central and local level.

5. Standardizing methods for the geographical assessment of the risk of BT/AHS/EHD spread in the Mediterranean basin and Europe in general with several tasks such as the development of the transmission pathways from currently affected areas, the review of published models that can be used for the assessment of infection probabilities of specified regions, the validation of risk assessment using case studies in selected countries (Algeria, Tunisia, Morocco, Turkey and Bulgaria) and the development of risk-based surveillance approaches including risk factors for increased probability of infection in specified populations and/or regions.

6. Dissemination of the results of the coordination action both by internal dissemination (annual reports delivered to the European Commission, accessible website with the sequences database, vector distribution and protocols, diagnostic procedures and surveillance protocols) and external dissemination (website, link to national and international information sources, synthesis on updated BT, AHS and EHD situation in the region).

VBD 21: MONOCLONAL ANTIBODY COMPETITIVE ELISA TEST TO DETECT ANTIBODIES AGAINST WEST NILE VIRUS IN EQUINE SERA

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IZSLER¹; Istituto G Caporale²

Key words: West Nile virus, Mab-based competitive ELISA, equine, antibody detection

West Nile virus (WNV) is a single strand RNA virus of the genus Flavivirus. It is included within the Japanese encephalitis virus serocomplex and is maintained and amplified in the environment through a natural transmission cycle which involves birds and ornithophilic mosquitoes. When mammals and, in particular, humans and horses, are implicated in the cycle, they act as incidental or dead-end hosts. However, in horses and humans WNV might cause fever and encephalitis. In Italy, WNV re-emerged after ten years in 2008, and over the last two years it has been identified in humans, horses, birds and mosquitoes. Horses are an important species useful for both passive and active surveillance. For the latter activity, good and accurate serological assays play a crucial role. The active surveillance in equidae, based on antibody detection, is considered an essential issue because they can act as sentinel species for infection.

In this paper, we developed and validated a monoclonal antibody based ELISA test to detect antibodies against WNV (WN-Ab ELISA) in equine sera using the ROC (Receiver Operating Characteristic) curves approach. This ELISA is a solid phase competitive assay which uses a specific WNV monoclonal antibody (Mab) 3B2 previously produced and characterized (1). This neutralizing Mab recognizes a conformational epitope located into domain III of the E protein and does not show cross-reactions against other flaviviruses (1).

Nunc Maxisorp micro-plates were coated with the 3B2 capture MAb. WNV inactivated with βpropiolactone was used as antigen. It was trapped by the coated MAb before being presented to the reaction with test sera. Serum samples were tested at two dilutions (1/5 and 1/10) and then the same MAb conjugated was added without washing, so that the competition was evaluated between serum antibodies and conjugated MAb. Results were calculated by determining the absorbance value reduction, expressed as % of inhibition with respect to the reference value (100% control wells). A total of 630 equine sera were tested in parallel using WN-Ab ELISA and serum-neutralization (SN) assay, which is considered as gold standard test. According to results of SN test, these sera were classified in 233 positive and 397 negative samples. The ROC curve with ELISA outcomes was constructed to enable the selection of optimal cut-off value and estimate the diagnostic sensitivity (Se) and specificity (Sp). The shape and the relevant "Area Under Curve" (AUC) values (0.996) showed a high test accuracy with very good Se (98.4%) and Sp (98.5%). The cut-off value representing the optimal balance of Se and Sp was 65%.

These results proved excellent diagnostic performances of the WN-Ab ELISA combined with ability to detect neutralizing antibodies specific to WNV. Compared to SN test, this competitive ELISA presents mayor advantages such as suitability for automation, low dependence to individual skills, spectrophotometric reading and easy interpretation of the results. Moreover, this assay can potentially be exploited to detect antibodies against WNV in different animal species.

References

Lelli D. et al. 4th Annual meeting EPIZONE, 7-10 June 2010, S. Malò France, pp161

VBD 22: NORDIC CLIMATE CHANGE: DATA FOR MODELING VECTOR BORNE DISEASES

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VET-DTU¹

Key words: Nordic; Climate; Diseases; Vector borne; Modeling;

The distribution of vector species is generally restricted by a range of different climatic and geographical factors, while the development and spread of the vector-borne diseases (veterinary and zoonotic) is often primarily temperature driven. Thus temperature and its derivatives are key factors in the modelling of vector-borne diseases. This puts a high demand on the quality and accuracy of the temperature data to be used as input in such models. In order to best capture the local temporal and spatial variation in the temperature surfaces, accurate daily temperature data were used in the present project.

Temperature data for a 30 year period (1980-2009) were obtained directly from the Meteorological stations in the five Nordic countries. The temperature data consisted of daily min and max measurements from 200 climate stations, adding up to more than two million measurements. Temperature point-data were interpolated to daily climate surfaces, using a squared IDW method. In the absence of a more local lapse rate the generally accepted lapse rate of -0.006 C°/m was used to account for the relationship between temperature and altitude. The interpolation was carried out on temperatures at sea-level and subsequently adjusted for the altitude. As a spherical adjustment, the min and max temperature was interpolated on a grid with a spherical surface geometry. This ensures a more accurate estimate of the temperature isolines in the northernmost areas (above the Arctic Circle) of Scandinavia. Various temperature derivatives were calculated in order to assess the geographical and seasonal variation in the area.

In order to evaluate the response of vector borne diseases to possible future climate changes and the subsequent potential spread into new areas, daily temperature predictions (mean, min and max) for three 20-year periods and 7 different prediction models were obtained from the Danish Meteorological Institute (DMI). Predicted temperature scenarios for year 2040 and 2060 were calculated and the data were incorporated in various models.

Additionally, major geographical, topographical, husbandry and biological spatial parameters relevant to the distribution of vectors were included in the database and used as input in various distribution models.

All collected datasets were assembled in a gridded climate database and presented at the website, www.nordrisk.dk. The website was created with the purpose of presenting the data to the public and making the data available to research projects in the Nordic countries. The website consists of an interactive web-application linked to a summarized climatic database. This allows for interactive selection of summary data for display. Detailed data files are available for research projects on request.

VBD 23: PHARMACOLOGICAL REACTIVATION OF EQUINE INFECTIOUS ANAEMIA VIRUS IN NATURALLY INFECTED MULES: CLINICAL, HAEMATOLOGICAL AND SEROLOGICAL RESPONSES

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Key words: Equine infectious anaemia, mules, immunosuppression

Equine infectious anemia (EIA) is a viral vector borne disease of equids even if epidemics have been traced to the multiple use of hypodermic needles and to the injection of substances contaminated with blood.

In Italy, since 2007 an extraordinary surveillance programme for the control of EIA was implemented due to a series of important outbreaks which had occurred over a short period in the spring of 2006. The programme imposes the serological control of all horses, donkeys and mules present in Italy, with the exception of those reared for human consumption. During each of the three annual campaigns till now held, a marked higher seroprevalence was observed in mules, even if the numeric consistency of this population was conspicuously inferior when compared to that of the donkey and horse population. The numerous cases of EIA registered in these animals have until now never been reported in surveillance programmes held even in other countries. In view of this and also due to the limited literature available regarding EIA in mules, epidemiological, etiological and clinical studies of the infection in these animals have been undertaken to better understand their role in the persistence and spread of this infection. For this, a study was conducted to evaluate the clinical, haematological and serological response of immune suppressed EIA infection in eleven naturally infected mules. The clinical signs for which the animals were controlled during the whole observation period, starting from 7 days prior to the 1st day of pharmacological immunosuppresion to 28 days later, were those typically occurring during an EIA infection in horses: rise in body temperature, thrombocytopenia. Other clinical signs for which the mules were monitored were alteration of their general condition, oedema, anaemia and congestion of the ocular and buccal mucosa, petechiae and jaundice. Biological samples for haematological and serological analyses, represented by blood, with and without anticoagulant, were collected daily for the whole experimental observation period. The serological methods used in the study were the following: an in-house C-Elisa, the Agar Gel Immunodiffusion Test (Agid) and the Immunoblotting (IB) while an automated counter Cell-Dyn 3700 (ABBOTT) was used to determine the platelet (PLT) counts.

The pharmacological immune suppression resulted in fever and/ or thrombocytopenia of eight mules. Of these, four mules also registered an increase of the serological response to the EIA virus. No direct correlation was observed between the clinical and haematological response and the serological reactivity. This study has been conducted to obtain information on the potential epidemiological role of these animals in the diffusion of the virus both during the chronic/unapparent and the acute/viremic phases of the EIA infection. The study of the genomic and antigenic characteristics of the viral strain infecting each animal in consideration of the different serological and clinical pattern observed for each mule and also in view of the fact that they came form different outbreaks, even if correlated geographically, might better explain some aspects till now unclear.

This study is the first report of the pharmacological reactivation of the EIA infection in naturally infected mules which apparently induces a mild to unapparent clinical form characterised by fever, thrombocytopenaia and an increase in serological reactivity only for some of the experimental animals.

VBD 24: PRELIMINARY STUDY OF OCCURRENCE OF WEST NILE VIRUS IN POLAND USING SEROLOGICAL EXAMINATIONS

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NVRI¹

Key words: serological test, neuroinfections, WNV

Introduction

West Nile virus (WNV) is an arbovirus belonging to the Flaviviridae family, genus Flavivirus, and is a member of the Japanese Encephalitis virus (JEV) serocomplex. It is an ssRNA + virus with a single open reading frame between 11,000 -12,000 nucleotides long flanked by a conservative terminal regions. A group of WNV neuroinvasive strains are neurotropic, proliferating in the central nervous system and inducing encephalitis or meningoencephalitis. WNV circulates from mosquitoes to birds and back to mosquitoes, but can be also transferred to mammals, including humans. The main virus vectors are mosquitoes from Culicidae family, especially Culex pipiens, and Culex molestus. A secondary role is also played by ticks from Hyalomma argas and Ornithodoros species. WNV can cause serious neurological disorders such as meningitis and meningoencephalitis in birds, mammals especially horses and humans.

Aim of study

The aim of this study was to determine the possibility the occurrence of West Nile virus antibodies in serum samples derived from the patients of Clinic of Neuroinfections and Infectious Diseases, Medical University in Bialystok, Poland.

Material and methods

Serum samples acquired from the twenty-four patients with the neurological symptoms such as meningitis, meningoencephalitis. The patients were hospitalized between May and September 2010 with diagnosis tick borne encephalitis and the lymphocytic inflammation. The highest mosquito activity was in this time. Additionally for the control we examined twelve horse serum samples derived from Reference laboratory of West Nile, AFSSA France.

As the diagnostic examinations we used the West Nile Virus Screen Competition ID-VET test both for horses and human according to the manufacturers protocol. The serum samples have been frozen in – 20°C before the examination in the BSL3 + laboratory. After the serum samples thawed the protocol of the WNV competition test was performed.

Results

According the ID-VET kit procedure 14 sera were positive from patients which early diagnosis was lymphocytic inflammation or tick borne encephalitis. One of the serum was doubtful and 9 were negative for West Nile virus antibody examinations. In control 6 positive results in serum samples from horses, one serum samples was doubtful and 5 serum samples were negative.

Conclusions

West Nile virus have not been detected in Poland using the PCR and Nested PCR method until now. But the serological examinations showed that there is the high possibility that the virus is present in our climate zone regarding the positive results in serum samples of hospitalized patients in Bialystok.

VBD 25: PROTECTIVE CELLULAR AND HUMORAL RESPONSES ELICITED BY A COMBINATORIAL PRIME-BOOST VACCINATION STRATEGY AGAINST BTV

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Key words: Bluetongue, recombinant vaccine, cellular and humoral responses

Bluetongue virus (BTV) is an insect-transmitted orbivirus that causes hemorrhagic disease in domestic and wild ruminants. Classical inactivated vaccines are effective to prevent BTV infection but do not allow the differentiation between naturally BTV infected and vaccinated animals. Recombinant DNA technology has allowed the development of novel approaches to design safe marker vaccines against infection. With this aim, DNA (pcDNA3) and recombinant modified vaccinia virus Ankara (rMVA) vectors expressing BTV-4 proteins have been engineered in our laboratory. Both neutralizing antibodies and cytotoxic T lymphocytes have a role in protective immunity against BTV. In order to find a vaccination strategy to improve the cellular response, DNA and rMVA expressing VP2, VP7, NS1 proteins of BTV-4 were generated.

C57BI/6J mice were immunized by heterologous prime-boost vaccination with DNAs/ rMVAs-VP2-VP7-NS1 administered 2 weeks apart. Ten days after the second immunization, sera and spleens were collected. The sera were used to quantify cytokine and neutralizing antibodies. Whole splenocytes were stimulated with recombinant VP2, VP7 and NS1 to analyze CD4+ and CD8+ T cell recall responses by intracellular cytokine staining and flow cytometry.

The results indicated that the heterologous prime boost vaccination with DNA and rMVA expressing VP2, VP7 and NS1 proteins of BTV-4 stimulated specific CD8+ T cell responses against these three BTV proteins. In addition, prime-boost vaccination of type I IFN receptor-deficient mice (IFNAR(-/-), an animal model for BTV infection established in our laboratory) with the same antigens protected against lethal challenge with BTV-4. Although additional characterization in the BTV natural host will be necessary, data from the IFNAR(-/-) mice model suggest that the DNA/rMVA-VP2,-VP7, NS1 marker vaccine is a promising alternative to inactivated BTV vaccines.

VBD 26: RESCUE OF 'SEROTYPED' BLUETONGUE VIRUSES

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CVI^1

Key words: Bluetongue reverse genetics Toggenburg TOV

Since 1998, European countries suffering by circulation of several of serotypes of bluetongue virus (BTV), like 1, 2, 4, 8, 9, and 16. The presence of new BTV serotypes have been reported at the border of Europe, in Israel and Morocco. To control BT-outbreaks vaccination with inactivated and/or modified-live vaccines have been carried out. However, it have taken several years before vaccine was available in sufficient amounts. In 2006, as an example, BTV8\net06 (IAH collection nr. BTV-8 NET2006/04, Maan et al., 2008) invaded North-Western Europe resulting in the largest BT-outbreak ever recorded. A mass vaccination campaign could only be launched in 2008, after inactivated BTV8 vaccine was available as produced by several companies, but still without full license or registration dossier.

Bluetongue is an arthropod-borne disease; transmission of BTV to ruminants, occurs by bites of species of Culicoides. BTV, family Reoviridae, genus Orbivirus, contains ten double stranded RNA segments encoding at least ten viral proteins. The BTV-serogroup consists of at least 24 serotypes. The serotype of BTV is dominantly represented by the highly variable VP2 and t lesser extend by VP5. These two proteins are closely interacted and form the outer shell of the virus particle. CryoEM density maps also reveal few, weak interactions between VP5 trimers and VP2 trimers and the underlying VP7 trimer. Above all, these proteins induce neutralizing antibodies in the ruminant target, and are mainly responsible for the protection against the respective BTV-serotype.

Recently, we have developed reverse genetics for several serotypes of BTV, including for vaccine strain BTV6\net08 (IAH-collection nr.: BTV-6 NET2008/05, Maan et al., 2010)(van Gennip et al., 2011). Here, we used this technology to generate 'synthetic' reassortants or synthetic 'serotyped' vaccine viruses for several serotypes of concern for Europe, and more exotic members of the BTV serogroup. Open reading frames or entire genome segments encoding VP2 and VP5 were cloned between the T7 promoter and a unique restriction enzyme site for run-off transcription. After RNA synthesis and transfection to BSR-monolayers, 'serotyped' BTV reassortants with different outer shells were rescued. Thus, these reassortants contain eight common genome segments originating from vaccine virus BTV6\net08 and differ in the genome segments S2 and S6 representing the serotype of the newly derived vaccine viruses. This approach opens the way to regenerate serotyped vaccine viruses for other, i.e. European, serotypes. Animal trials are planned to study the efficacy and safety of these serotyped vaccine viruses. In order to meet safety issues, these serotyped viruses can be used as production viruses for inactivated vaccines. In the view of preparedness on future BToutbreaks, master seeds of serotyped production viruses for all BTV-serotypes will significantly reduce the time between BTV-introduction and emergency vaccination.

- Maan, et al., 2008. Virology 377, 308-318.
- Maan, et al., 2010. PlosOne 5, 1-17.
- van Gennip et al., 2011 in preparation, and presentation/poster.

VBD 27: Reverse genetics for recent field strains of bluetongue virus

van Gennip, René G.P.¹; van de Water, Sandra G.P.¹; Veldman, Daniel¹; Wright, Isabel M.²; Potgieter, Christiaan A.²; <u>van Rijn, Piet A.¹</u>

CVI¹; Deltamune²

Key words: Bluetongue reverse genetics vaccine

Bluetongue virus (BTV), family Reoviridae, genus Orbivirus, contains ten double stranded RNA segments encoding at least ten viral proteins. Bluetongue (BT) is an arthropod-borne disease; transmission to ruminants, including cattle, sheep, and goats, occurs by bites of species of Culicoides. Since 1998, BTV serotypes 1, 2, 4, 9, and 16 have invaded European countries around the Mediterranean Basin. In 2006, BTV8\net06 (IAH collection nr. BTV-8 NET2006/04, Maan et al., 2008) invaded North-Western Europe resulting in the largest BT-outbreak ever recorded. BTV research has a long record (reviewed by Roy 2005), but molecular virology studies desperately needs methods to genetically modify BTV to study features in more detail. Recently, a genetic modification system for bluetongue virus based on uptake of synthetic genome segments was developed, and was used to isolate several reassortants of BTV6 and BTV8 (van Gennip et al., 2010). BTV-rescue with mutated genome segments was expected to be more difficult due to less viability of these mutant BTVs in the presence of wild type BTV. Classical reverse genetics has been published for BTV1 (Boyce et al., 2008), but reverse genetics is not published for virulent BTV, such as BTV8\net06 and other related orbiviruses. The first attempts to develop reverse genetics for BTV8\net06 based the published sequence (Maan et al., 2008) have failed. Extensive sequencing studies were performed on BTV8\net07 (IAH collection nr. BTV-8 NET2007/01) from a 1st passage on embryonated eggs, BHK21 and KC cell cultures and directly from blood. All ten complete genome segments were reversely transcribed, PCR-amplified (Potgieter et al., 2009), and sequenced with 454 technology (Roche GS FLX technology). Generated sequences were compared with published data to determine the consensus sequences. Subsequently, previously derived cDNAs based on the published sequences were corrected with respect to the consensus amino acid sequences. In parallel, reverse genetics for BTV1 (Boyce et al., 2008) was reproduced, and optimized. Therefore genome segments were cloned under control of the T7 RNA-polymerase promoter and were flanked by a restriction enzyme site at the 3'-terminus. Capped positive stranded run-off RNAs were synthesized in vitro. BSR-monolayers were transfected twice with mixtures of purified in vitro synthesized RNAs. The mixture of the first transfection contains positive sense capped RNAs of six segments, whereas after 18 hours all ten RNAs were transfected. A similar approach was followed to develop reverse genetics for virulent BTV8\net06, and nonvirulent BTV6\net08. For the latter, the consensus sequence was determined as described above (Maan et al., 2010). In summary, classical reverse genetics was developed for cell-adapted BTV1, virulent BTV8\net07, and nonvirulent or vaccine strain BTV6\net08. The presented reverse genetics for BTV is very promising as genetic modification system as demonstrated in several presentations/posters. These results further show the feasibility of genetic modification of related orbiviruses, like African horse sickness virus and enzootic hemorrhagic disease virus.

- Boyce et al., 2008. J.Virol. 82, 8339-48.
- Maan, et al., 2008. Virology 377, 308-318.
- Maan, et al., 2010. PlosOne 5, 1-17.
- Potgieter et al., 2009. J.Gen.Virol. 90, 1423-1432.
- Roy, 2005. Adv. in Virus Research 64, 69-123.
- van Gennip et al., 2010. BMC Virology Journal 7, 261-267.

VBD 28: Safety of a bivalent BTV-1/BTV-8 inactivated vaccine in young lambs

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MERIAL^1

Key words: BTV, sheep, vaccine, safety, young

Introduction

Bluetongue outbreaks have seriously affected the cattle and sheep industries in Europe. Since 2008, large-scale vaccination programmes using inactivated vaccines have been conducted in Europe to control BTV infections, notably against BTV-8. Inclusion of young animals in the vaccination programme is not only essential for the success of a BTV control strategy but also to ensure safe animal movements. Consequently, safety of a vaccine to be used in young animals is of great importance for farmers' acceptance of the vaccination strategy. To evaluate vaccine safety, which is freedom from undesirable side-effects, vaccine registration bodies generally ask for data regarding the administration of an over-formulated vaccine. This overformulated vaccine is administered as a "double dose" and a "repeated dose". In this paper, we describe the set up and the results of a safety study in young lambs using an over-formulated BTV-1/8 bivalent vaccine of the BTVPUR ALSAP range (Merial).

Material and methods

Twenty BTV sero-negative one-month old lambs were randomly allocated to 2 groups of 10 animals each, on the basis of their bodyweight. One group was subcutaneously vaccinated whereas the other group served as control. On day 0 (D0), animals belonging to the vaccinated group were injected with 2 mL ("double dose") of an over-formulated inactivated bivalent BTV-1/8 vaccine (BTVPUR ALSAP, Merial). Fourteen days and 29 days later the lambs were again vaccinated with 1 mL of the same vaccine ("repeated dose"). The control group remained unvaccinated.

Monitoring of the animals included:

- individual clinical examination,
- recording of rectal temperature for 4 days following each injection,

- monitoring of local reactions until D49 with subsequent histological analysis of the injection sites, and

- recording of bodyweight gain.

Results

The safety of the administration of an overdose (D0) and repeated administration of one dose (D14 and D29) of the bivalent BTV-1/BTV-8 over-formulated vaccine tested was demonstrated in one-month old lambs with:

- moderate and transient temperature increase following the administration of a repeated dose,

- no treatment-related general reaction,

- limited to moderate swelling reactions at the injection sites that had disappeared or were of very limited size on D49,

- classical local subcutaneous foreign-body-like lesion at histology, and

- no impact on bodyweight gain.

CONCLUSION

The use of an inactivated bivalent BTVPUR ALSAP vaccine is safe in young lambs and did not impact the daily weight gain.

BTVPUR ALSAP is a registered trademark of Merial.

VBD 29: SAFETY OF A BIVALENT BTV-4/BTV-8 INACTIVATED VACCINE IN YOUNG CALVES

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MERIAL¹

Key words: BTV, bovine, vaccine, safety, young

Introduction

Bluetongue outbreaks have seriously affected the cattle and sheep industries in Europe. Since 2008, large-scale vaccination programmes using inactivated vaccines have been conducted in Europe to control BTV infections, notably against BTV-8. Inclusion of young animals in the vaccination programme is not only essential for the success of a BTV control strategy but also to ensure safe animal movements. Consequently, safety of a vaccine to be used in young animals is of great importance for farmers' acceptance of the vaccination strategy. To evaluate vaccine safety, which is freedom from undesirable side-effects, vaccine registration bodies generally ask for data regarding the administration of an over-formulated vaccine. This overformulated vaccine is administered as a "double dose" and a "repeated dose".

In this paper, we describe the set up and the results of a safety study in young calves using an over-formulated BTV-4/8 bivalent vaccine of the BTVPUR ALSAP range (Merial).

Material and methods

Twenty BTV sero-negative calves one month of age or less were randomly allocated to 2 groups of 10 animals each, on the basis of their bodyweight. One group was subcutaneously vaccinated whereas the other group served as control. On day 0 (D0), animals belonging to the vaccinated group were injected with 2 mL ("double dose") of an over-formulated inactivated bivalent BTV-4/8 vaccine (BTVPUR ALSAP, Merial). Fourteen days and 28 days later the calves were again vaccinated with 1 mL of the same vaccine ("repeated dose"). The control group remained unvaccinated.

Monitoring of the animals included:

- individual clinical examination,
- recording of rectal temperature for 4 days following each injection,

- monitoring of local reactions until D49 with subsequent histological analysis of the injection sites, and

- recording of bodyweight gain.

Results

The safety of the administration of an overdose (D0) and repeated administration of one dose (D14 and D28) of the bivalent BTV-4/BTV-8 over-formulated vaccine tested was demonstrated in one-month old calves with:

- very moderate and transient temperature increase following the administration of a repeated dose,

- no treatment-related general reaction except very transient apathy or decrease of appetite in one calf,

- limited swelling reactions that had disappeared within 4 weeks after vaccination,
- granulomatous inflammatory reactions of very limited size in one third of the injection sites,
- no impact on bodyweight gain.

Conclusion

The use of an inactivated bivalent BTVPUR ALSAP vaccine is safe in young calves and did not impact the daily weight gain.

BTVPUR ALSAP is a registered trademark of Merial.

VBD 30: SURVEILLANCE ON TBEV IN TICKS COLLECTED ON HUNTED WILD ANIMALS IN LOMBARDIA REGION (ITALY) – PRELIMINARY RESULTS

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IZSLER¹

Key words: TBE, ticks, wild fauna, Italy

Background

The origins of emerging infectious diseases are correlated with socio-economic, environmental and ecological factors. Tick-borne viruses can rise or appear in areas where diseases were not previously detected. Tick-borne encephalitis (TBE) in Europe is a disease caused by tick-borne encephalitis virus (TBEV, genus Flavivirus, family Flaviviridae) and is maintained in cycles involving Ixodid ticks (Ixodes ricinus) and wild vertebrate hosts. Tick-borne encephalitis (TBE) is a severe disease that has been endemic in north-east Italy since 1992. Over the past two decades, there has been an increase in the number of human cases reported in Italy, mainly in the nort-east Alp region. The aim of this study is to investigate the presence of TBEV by Real Time PCR in ticks collected on wilds animals collected in areas near this region but where no TBE cases were reported.

Methods

Ixodid ticks were collected from roe deer (Capreolus capreolus), red deer (Cervus elaphus), chamois (Rupicapra rupicapra) red fox (Vulpes vulpes), hedgehog (Erinaceus europaeus), badger (Meles meles) and European brown hare (Lepus europaeus). Ticks were removed and identified following taxonomic standard keys. Nucleic acids were analyzed from pools of immature stage collected on the same animal and from individual adult ticks. RNA was extracted using Trizol®LS Reagent (Invitrogen, Carlsbad, CA). cDNA synthesis was achieved using random examer (Roche Diagnostics, Mannheim, D) and SuperScript® II Reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The research of TBE virus was carried out by using Real Time PCR with primers and probe described by Schwaiger e Cassinotti (2003).

Results and Conclusions

A total of 288 ticks were tested for TBEV presence. Sixty-two wild animals were collected from six provinces of Lombardia region. I. ricinus is the dominant species in woodland in Lombardia region (84% of tick sampled), followed by Ixodes canisuga (8% n=24), Ixodes hexagonus (n=18; 6%) and Dermacentor marginatus (n=4; 2%).

TBEV was not detected during this survey, while the virus is known to be present in some area of North-Eastern Italy, including the neighbouring region Veneto. The presence and the ecology of the TBEV specific vector and its wide variety of hosts suggests a possible introduction of the virus in Lombardia and more extensive survey has to be conducted to assess this risk.

VBD 31: THE HIGHLY CONSERVED INTEGRIN BINDING MOTIF RGD IN VP7 OF BLUETONGUE VIRUS IS NOT ESSENTIAL

VAN GENNIP, RENÉ G.P.¹; VAN DE WATER, SANDRA G.P.¹; <u>VAN RIJN, PIET A.¹</u>

CVI^1

Key words: Bluetongue VP7 vaccine development

Bluetongue virus (BTV), family Reoviridae, genus Orbivirus, contains ten double stranded RNA segments encoding at least ten viral proteins. Bluetongue (BT) is an arthropod-borne disease; transmission to ruminants, including cattle, sheep, and goats, by bites of species of Culicoides. Three layers of proteins could be recognized in the virus particle. The inner shell or sub-core consists of VP3, and is surrounded by VP7 forming the core particle. The outer shell consists of structural proteins VP2 and VP5 representing the serotype. BTV multiplies in all kinds of ruminants and specific Culicoides species, and consequently can infect both mammalians as well as insect cells. Due to the neutralizing capacity of antibodies directed against the outer shell, it is believed that the outer shell is involved in attachment to the mammalian cell. In contrast, VP7-directed antibodies are not neutralizing in mammalian cell cultures, but could neutralize core particles. Further, digestion of African horse sickness virus, another serogroup of orbiviruses, with equine serum proteases resulted in increased infectivity for insect cells (Mertens et al., 1996). These data suggests an important role for VP7 in infection of insect cells.

BTV-protein VP7 could be divided into three parts, an N-terminal bottom domain, a centrally located top domain, and a C-terminal bottom domain. VP7 forms the outer core of the core particle of orbiviruses. The top domain of VP7 of all serogroups of orbiviruses contain the integrin binding motif RGD. Although the precise location is not conserved among the serogroups, the motif itself is highly conserved. Binding studies with baculovirus expressed core particles of VP3 and VP7 of with mutants in the motif have a reduced affinity for cell lines of Culicoides (Boon-Huan et al., 2001). Here we have studied the role of the RGD motif in infectious virus. Several mutations were introduced in the motif, and mutant BTVs were rescued by reverse genetics (van Gennip et al., 2011). Although BTV-mutants were viable, some mutations resulted in a lethal phenotype. These results demonstrate that this region plays an important role in BTV-replication. However, effects on the structural functions of VP7, like interactions with the outer shell and the sub-core cannot be excluded. Even more, VP7protein with a deleted Arginine residue could not be immunostained by BTV-sera and monoclonal antibodies. This suggests that deletion of the Arginine results in a completely changed structure or misfolding of the top domain of VP7. Subsequently, it is likely that interactions with structural proteins is abolished. We suggest that the structural functionality of the other VP7-mutants could also be disturbed, but still resulted in detection by sera and monoclonal antibodies. For viable mutant BTVs, the effects on growth characteristics on BSRcells and KC-cells will be determined, and discussed.

- Mertens et al., 1996. Virology 217, 582-593.
- Boon-Huan et al., 2001. JVirol. 75, 3937-3947.
- van Gennip et al., 2011. in preparation, and poster/presentation.

VBD 32: THE NETHERLANDS STRAIN OF BTV SEROTYPE 8 IN WHITE-TAILED DEER

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USDA Agriculture Research Services¹; USDA APHIS²; CVI³; Colorado State University⁴

Key words: Bluetongue white-tailed deer disease risk

Bluetongue virus (BTV), family Reoviridae, genus Orbivirus, contains ten double stranded RNA segments encoding at least ten viral proteins. Bluetongue (BT) is an arthropod-borne disease; transmission to ruminants, including cattle, sheep, goats, and deer species by bites of species of Culicoides. In 2006, BTV8\net06 (IAH collection nr. BTV-8 NET2006/04, Maan et al., 2008) invaded North-Western Europe resulting in the largest BT-outbreak ever recorded. This BTVstrain differs from other BTV-strains in many aspects; competent vector(s), transplacental and oral transmission, and severity in cattle (Meiswinkel et al., 2007, Dijkstra et al., 2008, Backx et al., 2007, 2009). To determine the susceptibility of USA white-tailed deer (Odocoileus virginianus) to the European strain of BTV8\net07 (IAH-collection BTV-8 NET2007/01), eight BTV-seronegative deer were injected subcutaneously in the neck and intradermally in the inner left leg. Two deer were sham inoculated to serve as uninfected controls and housed with infected animals to verify the inability of this virus to spread by direct contact transmission. Body temperatures and clinical signs were recorded daily. Sequential blood samples were analysed for BTV RNA with qRT-PCR, for BTV serum antibodies by cELISA, and for infectious virus by plaque assay. At necropsy, tissue samples were taken for histopathological examination and tested by qRT-PCR for viral RNA. Deer developed moderate to severe clinical disease from 8 to 15 days post inoculation (dpi). Peak vireamia by qRT-PCR was from 7-10 dpi with detectable virus titres observed up to 28 dpi in some deer. Antibody titres were detected by cELISA starting at day 6, peaked by day 10, and continued through the end of the experiment (day 28). These results suggest that if BTV8 is accidentally or intentionally introduced into the USA, considerable disease would be expected in USA white-tailed deer and they would serve as significant virus reservoirs.

- Backx et., 2007 Vet. Record 161, 591-593.
- Backx et al., 2009 Vet. Microbiol. 138, 235-243.
- Meiswinkel et al., 2007 Vet. Record, 161, 564-565.
- Dijkstra et al., 2008 Vet. Record. Letter 422.

VBD 33: VIABLE 'SYNTHETIC' REASSORTANTS OF BLUETONGUE VIRUS CONTAINING GENES OF TOGGENBURG ORBIVIRUS (TOV)

VAN RIJN, PIET A.¹; VAN DE WATER, SANDRA G.P.¹; MARIS-VELDHUIS, MIEKE A.¹; VAN GENNIP, RENÉ G.P.¹

CVI^1

Key words: Bluetongue Toggenburg TOV reverse genetics

Bluetongue virus (BTV) belongs to the family Reoviridae, genus Orbivirus in which many serogroups are present. Serogroups are divided by their capability to exchange genome segments, a phenomenon known as reassortment. Each serogroup contains many serotypes as defined by minor or no cross neutralization varying from a single member up to 24 or more serotypes for the BTV serogroup. Recently a new orbivirus isolated from goats has been identified, and was designated Toggenburg orbivirus (TOV) (Hofmann et al., 2008). TOV is genetically closely related to BTV, and is therefore proposed as 25th serotype of the BTV serogroup. Despite extensive attempts by several research groups, TOV could not be cultured in all kind of cell lines so far. Consequently, cross neutralization tests of TOV with reference BTV sera cannot be performed. On the hand, sera induced by TOV did not neutralize any of the 24 defined BTV-serotypes. Further, reassortment events between BTV and TOV cannot be proven in the laboratory setting. Thus, research on TOV has tremendously hampered by lack of culturing of TOV. Recently, we have developed reverse genetics for several BTVs (van Gennip et al., 2011). Here, this technology was successfully used to rescue 'synthetic' BTVreassortants containing on or more genes originating from TOV. Therefore, cDNAs of open reading frames of TOV were cloned between non-translated regions of BTV. On their turn, These cDNAs of complete genome segments were cloned between the T7-promoter and a unique restriction enzyme recognition site for run-off RNA synthesis. Combination of sets of S1-S10 were used to rescue BTV/TOV reasortants. For example, 'synthetic' reassortants were rescued with combinations of VP2, VP5, VP7 and NS3/NS3a of TOV, concluding that the tested TOV-genes encode functional orbiviral proteins. However, rescue of 'synthetic' reassortant with TOV-VP2 depended on co-expression of TOV-VP5. BTV/TOV-reassortant expressing all four proteins, VP2, VP5 VP7 and NS3/NS3a of TOV, was also infectious. These results strongly support the proposal that TOV is indeed a member of the BTV serogroup. Further, tested proteins did not appear the cause of the complete failure to culture TOV. Though, all BTVreassortants expressing TOV-VP5 were harder to rescue, and therefore could decrease the efficiency of isolation of wild type TOV. The 'synthetic' BTV/TOV-reassortants will be studied in different cell lines. In addition, rescue of TOV/BTV-reassortants with other TOV-proteins is in progress. Recent results will be discussed.

- Hofmann et al., 2008. Emerg. Inf. Dis. 14, 1855-1861.
- van Gennip et al., 2011. in prep. and poster/presentation.

Diagnostics

D 1: A COMPARATIVE STUDY OF CSFV ANTIBODY LEVELS IN PIGS VACCINATED WITH THE CHIMERIC VACCINE CP7_E2GIF OR A C-STRAIN VACCINE

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Key words: DIVA, diagnostic, vaccination, CSFV, Chimeric vaccine

Classical swine fever (CSF) is a viral disease affecting both domestic pigs and wild boars worldwide. Outbreaks of CSF impair internal and international trade of pigs and pig products. Therefore, the disease is of major importance from an economic as well as welfare view. Effective live attenuated vaccines against CFSV are available and many of these provide lifelong immunity. However, the antibody response induced by these vaccines cannot be distinguished from that observed in naturally infected animals and therefore great efforts are currently focusing on the development of new DIVA vaccines allowing differentiation of infected from vaccinated animals.

Previously, the chimeric virus vaccine, CP7_E2gif, has been presented as a safe live DIVA vaccine candidate against CSF (Rasmussen et al., 2007). CP7_E2gif is unique as no CSFV sequence is present in the genome. Thus, the backbone consists of Bovine viral diarrhoea (BVDV) strain CP7 of which the envelope protein E2 has been replaced with E2 from Border disease virus (BDV) strain Gifhorn. Therefore, CSFV E2 specific DIVA detection is an option (Rasmussen et al., 2008; Rasmussen et al 2009).

The aim of the present study was to further evaluate the DIVA specificity of the chimeric pestivirus CP7_E2gif compared to the C-strain "Riems" conventional live attenuated vaccine. Two groups of 12 pigs were vaccinated by intramuscular injection with each of the 2 vaccines, respectively, and the serological response was measured twice weekly up to 28 days after vaccination. Furthermore, the pigs were monitored daily for general health status, clinical signs of disease and body temperatures. Necropsy with special focus on pathological changes, which could be linked to the vaccination, was carried out on 3 pigs every week from post vaccination day (PVD) 7 to 28. After vaccination, all 24 pigs remained healthy as neither clinical, pathological nor body temperature changes were observed. Seroconversion, measured by a routine blocking CSFV ELISA, could be observed from day 14 post vaccination in pigs vaccinated with C-strain. In contrast, CP7_E2gif vaccinated pigs were not tested antibody positive during the 28 days period. Further analyses for BDV and CSFV E2 specific antibodies in serum samples from the vaccinated pigs as well as for the presence of neutralizing antibodies against CSFV, BVDV and BDV are in progress and these results will be presented at the meeting.

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D 2: A GENERIC REAL-TIME TAQMAN ASSAY FOR SPECIFIC DETECTION OF LAPINIZED VACCINE STRAINS AGAINST CLASSICAL SWINE FEVER

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Key words: TaqMan assay, C-strain, DIVA, CSFV

Classical swine fever (CSF) is a highly contagious disease, causing heavy economic losses in the pig industry worldwide. Vaccination of pigs with lapinized vaccines is still practised in regions of the world, where the virus is present, in order to prevent and control the disease. However, a single real-time assay that can detect all commonly used lapinized vaccines, namely, Lapinized Philippines Coronel (LPC), Hog Cholera Lapinized virus (HCLV) and two cell culture adapted derivatives of HCLV, Chinese (C) strain and Riems strain is still lacking. The objective of this study was to develop a generic real-time RT-PCR assay for the specific detection of these lapinized vaccine strains.

Primers and a TaqMan probe were designed targeting the Npro gene region. The real-time TaqMan RT-PCR assay was performed using SuperScript III Platinum One-Step Quantitative RT-PCR. A total of 139 reference pestiviruses and RNA preparations were evaluated. This collection covered all major genotypes (1.1, 1.2, 1.3, 2.1, 2.2, 2.3, and 3.2). Furthermore, 23 tissue samples were obtained from an animal vaccination and challenge experiment and tested by the assay.

All wild type CSFV strains and pestiviruses were found negative for the C-strain by the assay that was performed in a RotorGene 3000 instrument. This indicated that the newly developed assay is highly specific for detection of lapinized vaccines, and that there is no cross-reactivity with CSFV from all ten genotypes nor with other pestiviruses. The assay had a detection limit of about 10 genome (Riems strain) copies per reaction, which was obtained readily from three independent tests. This indicated that the assay is highly sensitive. The assay had a reaction efficiency of 1.01, a slope value of -3.29, and a reaction coefficiency (R2) of 0.99. Testing of the LPC transcript showed a reduced sensitivity, detecting a lower limit of about 103 genome copies per reaction. The assay is also highly repeatable: the coefficiency of variation of Ct values in three runs was 2.77% for the detection of 10 copies of the vaccine viral RNA. In conclusion, this study provides a potentially useful tool for the specific detection of commonly used lapinized vaccines and the differentiation of these vaccines from wild type CSFV.

Eight tonsil samples and 15 retropharyngeal lymph nodes from unvaccinated animals, which were infected with either of the challenge strains, were negative, confirming the specificity of this assay to the vaccine strain. Tonsil samples from 12 vaccinated animals yielded positive results in 11 cases. The one negative sample was from a vaccinated animal that had to be euthanized early, as it was not protected from the challenge. In contrast, when retropharyngeal lymph nodes were tested vaccine virus was detected in only 12 of 37 vaccinated animals. These results were in accordance with results obtained using another method.

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D 3: APPLICATION OF A REAL-TIME REVERSE TRANSCRIPTION LOOP MEDIATED AMPLIFICATION METHOD TO THE DETECTION OF RABIES VIRUS IN ARCTIC FOXES IN GREENLAND

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Key words: Rabies, LAMP, Arctic-like, Cosmopolitan, fox

Reverse transcription loop mediated amplification (RT-LAMP) offers a rapid, isothermal method for amplification of virus RNA. In this study a panel of positive rabies virus samples originally prepared from arctic fox brain tissue was assessed for the presence of rabies viral RNA using a real time RT-LAMP. The method had previously been shown to work with samples from Ghana which clustered with cosmopolitan lineage rabies viruses but the assay had not been assessed using samples from animals infected with rabies from the arctic region. The assay is designed to amplify both cosmopolitan strains and arctic-like strains of classical rabies virus due to the primer design and is therefore expected to be universally applicable independent of region of the world where the virus is isolated. Of the samples tested all were found to be positive after incubation for 25 to 30 minutes. The method made use of novel enzymology from OptiGene but fluorescence reads were performed in a Stratagene MX instrument. The identity of the product was confirmed using melt analysis with all products melting at temperatures between 87.1C and 88.2C, similar to a rabies virus positive control. This demonstrates that rabies virus of arctic origin virus can be detected using RT-LAMP and the method reported is more rapid than the real-time RT-PCR. Further arctic fox samples are under analysis in order to confirm these findings.

D 4: CHARACTERIZATION OF THE RHDV CAUSING OUTBREAKS IN 2003 – 2010 IN RUSSIA

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Key words: RHDV; RT-PCR; real-time RT-PCR; VP60; phylogenetic analysis

Rabbit haemorrhagic disease (RHD) is a highly contagious disease, characterised by high morbidity and high mortality in both wild and domestic adult rabbits (Oryctolagus cuniculus). The genome of rabbit hemorrhagic disease virus (RHDV), a identified member of the family Caliciviridae (V. Ohlinger et al., 1990), consists of a single plus-stranded RNA of 7,437 nucleotides that has VPg attached covalently to the 5' end and is polyadenylated at the 3' end.

During last 2 years the number of RHD outbreaks in Russia increased dramatically. Thus, in 2008 only one RHD outbreak was reported in Krasnojarsky region, but since 2009 till 2010 the disease was reported from more than 15 regions of the country. Owing to this situation, definition of biological properties of isolates, obtained from RHD outbreaks during 2003-2010, appears to be an actual task. Detection of RHDV was performed using ELISA kit (I. Vishnyakov et al.,1992), RT-PCR and Real-Time RT-PCR (A. Gall et al., 2007). This study used isolates, obtained from RHD outbreaks during 2003-2010, and strain «V-87», which apply for production of inactivated vaccine in NRIVVaM.

All positive samples were tested by hemagglutination test (HA). It performed with human Group O red blood cells (RBCs).

Among RHDV isolates, obtained in Russia during 2003-2010 temperature-depending isolates, showing his hemagglutination activity at + (4±2) 0C (isolate Perm-10, strain Manihino-09), and temperature-depending isolates occurred. No non- hemagglutinating isolates were detected.

For nucleotide sequencing of genes VP60, 5 primers pairs were designed, which amplify overlapping gene fragments. Phylogenetic analysis showed, that strain «V-87» refer to original subtype of RHDV, and all analysed samples to RHDVa subtype. The analysis of nucleotide sequences of gene VP60 of Russian strains and isolate revealed high identity between them, ranged from 95% to 97,5%. Homology between RHDVa isolates and strain «V-87» ranged from 91% to 93%.

The results of presented studies demonstrate the quite high divergence rate in nucleotide sequence gene encoding VP60 of Russian RHDV strains and isolates.

D 5: COMBINING RANDOM ACCESS AMPLIFICATION AND MASSIVE PARALLEL SEQUENCING ON AVIAN INFLUENZA VIRUS GENOMES: TOWARDS COST EFFECTIVE SEQUENCE INDEPENDENT WHOLE GENOME DETERMINATION?

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Key words: avian influenza, next generation sequencing, molecular epidemiology

RNA virus sequence variability can pose a challenge to the determination of whole genomes. Whole genome sequencing protocols based on PCR amplification and Sanger sequencing may be subject to mutations in priming regions. As a result, avian influenza virus (AIV) whole genome sequencing methods remain limited to restricted groups (such as primer sets developed for H5N1 viruses) and the determination of whole genomes of less abundant AIV subtypes is often leading to extensive primer design and primer walking. Moreover, the typical coverage/quality of sequences (typically < 5x) for current consensus sequences in public databases may in itself offer only a restricted view on the RNA virus genome. An additional limitation of traditional amplification + sequencing protocols is that information of multiple infections or quasispecies may be lost due to preferential amplification of sequence variants. Massive parallel sequencing technologies were previously optimized on PCR amplicons representing H5N1 AIV genomes. In the context of metagenomic analysis of human influenza patients or quasispecies analysis of influenza A virus samples, this sequencing technology was already applied without nucleic acid pre-amplification bias, resulting in a high proportion of non influenza specific reads. We explore the use of optimized random access RNA amplification on enriched viral RNA in combination with massive parallel sequencing for the generation of whole genome sequences (experiment with 9 AIV genomes of various subtypes). We discuss the current pitfalls and advantages of this approach and the feasibility of cost efficient high quality AIV genome determination without prior sequence knowledge.

D 6: COMPARISON OF SPECIFICITY AND SENSITIVITY OF FOUR DIFFERENT RT-PCR ASSAYS DESIGNED FOR THE DIAGNOSTIC OF BOVINE RESPIRATORY SYNCYTIAL VIRUS

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Key words: BRSV, diagnosis, RT-PCR

Bovine respiratory syncytial virus (BRSV) is a member of Pneumovirinae subfamily of Paramyxoviridae family. It is one of the major causative agents of respiratory tract diseases in cattle and is responsible for high economical losses in cattle industry around the world. Detection of BRSV based on virus isolation in cell culture is problematic due to its high lability. Improper storage conditions and prolonged transport could make the virus undetectable. Therefore range of alternative diagnostic methods were designed. Among them RT-PCR is regarded as both highly specific and sensitive. However due to the high differences in variability between BRSV genes, assay results could depend on the choice of the targeted region.

In this paper four RT-PCR assays specific to different regions of BRSV genome were optimized and evaluated for their sensitivity and specificity. Primers used in this study were specific for genes coding 3 BRSV proteins: 2 sets of primers were specific to highly conservative nucleoprotein N, one set to highly variable glycoprotein G and one to glycoprotein F.

All of the assays were able to detect both of the BRSV strains used as the positive control however one of the assays specific to nucleoprotein N was not selective enough to distinguish HRSV from BRSV. None of the assays detected BPIV3. Sensitivity of the assays varied, ranging from 10^1,66 TCID50 to 10^3,66 TCID50. However this differences were probably not associated with the target gene and rather with the proprieties of particular primers and test conditions. Among tested assays, one specific to sequence of glycoprotein G gene, based on the B7:B8 primer pair, seemed to be the most useful for BRSV diagnostic, as it was characterized by both high sensitivity and specificity.
D 7: COMPARISON OF TWO H1N2 SWINE INFLUENZA A VIRUSES FROM DISEASE OUTBREAKS IN PIGS IN SWEDEN DURING 2009 AND 2010

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Key words: Influenza A, swine influenza virus SIV, genetic comparison

The influenza A virus subtypes H1N1, H1N2 and H3N2 are prevalent in pig populations worldwide. In the present study, two relatively uncommon swine influenza virus (SIV) H1N2 subtypes, isolated in Sweden in 2009 and 2010, were compared regarding their molecular composition and biological characteristics. The differences regarding markers purportedly related to pathogenicity, host adaptation or replication efficiency. They included a truncated PB1-F2 protein in the earlier isolate but a full length version in the more recent one; differences in the number of haemagglutinin glycosylation sites, including a characteristic human one; and a nuclear export protein with altered export signal. Of particular interest, the NS1 amino acid sequence of swine H1N2-2009 and 2010 has a 'unique or very unusual' PDZ binding domain (RPKV) at the C-terminal of the protein, a motif that has been implicated as a virulence marker. Concerning biological properties, these viruses reached lower titre and showed reduced cytopathogenicity in MDCK cells compared with an avian-like H1N1 isolate A/swine/Lidkoping/1193/2002 belonging to the same lineage as the 2009 and 2010 isolates. The findings should contribute to better understanding of factors related to the survival/extinction of this uncommon reassortant variant.

D 8: COWPOX VIRUS: CHARACTERIZATION OF AN OLD BUT EMERGING ZOONOSIS

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Key words: Orthopox, CPXV, zoonosis, rats, pathogenesis

Cowpox virus infection is a zoonosis in Europe, which mainly affects pet owners or veterinarians after contact to diseased pet or zoo animals. The risk of human infection is probably increasing in the last years as a consequence of the decreasing immunity against orthopoxviruses in the human population since smallpox vaccination has been terminated more than 30 years ago.

Most human cases of cowpox virus infection have been associated with infected cats, however, pet rats have become the second most common transmission host to humans in recent years. Interestingly, the young pet rats were severely ill and often died albeit wild rodent species are regarded to be the classical reservoir hosts of cowpox virus.

Here we report about the disease that has been experimentally induced in pet rats and outbred wistar rats using three different cowpox viruses isolated 2009 and 2010 as well as the reference strain "Brighton". Wistar rats and pet rats (one isolate) were infected with 104 or 106 TCID50, respectively. Viral excretion was continuously recorded by sampling oropharyngeal swabs. Additionally the genome load of a broad set of tissues was determined. Interestingly, only the cowpox virus isolated from a diseased pet rat induced severe clinical signs and lethal infection (high doses group) both in the wistar and the pet rats, whereas the reference strain Brighton, and isolates from a cat and an alpaca caused clinical signs only in a small proportion of inoculated animals. Furthermore, we infected cows with the "Brighton" strain or with the pet rat derived isolate. Intravenous injection ororonasal application and scarification of the teats were the inoculation routes, but typical cowpox lesions like blisters and crusts were detected only in 2 out of 4 cows after intravenous inoculation.

In order to link the differences in pathogenesis to potential virulence markers we also developed a protocol for full-length viral genome sequencing.

With the establishment of a standardised animal model and the possibility to prospectively use immunological tools, a more detailed in vivo characterization of different cowpox virus isolates is now possible. Identification of virulence markers by additional sequence comparison might be also beneficial for other human pathogenic orthopoxvirus infections (e.g. for monkeypox).

D 9: DETECTION OF ASFV BY NESTED REAL-TIME PCR

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Key words: ASFV, real-time PCR, diagnostics

African swine fever (ASF) is a devastating haemorrhagic fever of pigs with mortality rates approaching 100 per cent. It causes major economic losses, threatens food security and limits pig production in affected countries.

Since 2007, ASF is reported in southern part of Russia. The last outbreaks of the disease were in Volgrad, Astrahan, Rostov and Krasnodar.

For detection of ASFV genome in pork products, in infected pigs during the first stages of the disease and in contaminated fomites or pork wastes nested real-time PCR has been developed. For this purpose, two primers pairs and a FAM-labeled TaqMan probe, specific for 72 encoding gene, were designed. The PCR product, amplified using external primers, was used as a template in real-time PCR with internal primers and probe.

The comparison of sensitivities of conventional PCR, real-time PCR and nested real-time PCR was performed using series of tenfold dilutions of ASFV reference strain (6 lg HAD50/ml)viral DNA. The sensitivity of nested real-time PSR appeared to be 2 lg HAD50/ml higher than the sensitivity of other methods. After that we have checked the sensitivity of the assay using tenfold dilutions od DNA isolated from tissues, and again it was 2 lg HAD 50 higher than for real-time PCR assay.

The specificity of the assay were demonstrated using a panel of strains from different historical and geographical origins, and recent Caucasus isolates originated from Volgogdad, Astrahan and Rostov, and also negative blood or tissue samples from domestic pigs, as well as genomic material from other swine viruses. The positive or negative status of the samples has previously been identified by PCR and real-time PCR. The entire positive samples were confirmed positive by the present assay. Among the previously scored negative, discrepant positive samples were identified among the African field samples which were confirmed by sequencing of the real-time PCR amplicons.

Thus, the nested real-time PCR presents a high sensitivity and specificity, including the currently circulating isolates. The assay provides a sensitive and reliable molecular diagnostic tool for ASF diagnosis in pork products, in infected pigs during the first stages of the disease and in contaminated fomites or pork wastes.

D 10: DETECTION OF BOVINE FOAMY VIRUS INFECTION USING MEAT JUICE AND TISSUES OF SLAUGHTERED COWS

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Key words: BFV, diagnostics, meat juice

Foamy viruses are the least known retroviruses, however infections caused by FVs are widespread and were found in humans and many animals species like primates, cattle, cats and horses. Since bovine foamy virus (BFV) is highly prevalent within cattle population all over the world, special attention should be paid on its presence in different biological and medical materials of bovine origin. Recent data from BFV inoculated calves showed the presence of viral DNA in all examined organs, with the exception of nervous tissue. Furthermore, while zoonotic potential of BFV can not be excluded BFV, introduction of BFV into food chain should be seriously concerned. There are several methods to detect infections of BFV including serological testing and detection of viral DNA by PCR. In this work we demonstrated usefulness of detection of BFV-specific antibodies in meat juice of seropositive cows and successful application of nested-PCR to detect BFV DNA in muscles and other tissues of naturally infected cows.

32 BFV infected cows were selected by the examination of their sera using ELISA with recombinant Gag protein of BFV. These cows were then slaughtered and blood, muscles, spleen, liver, lung, lymph nodes, salivary glands and udder samples were collected. Meat juice samples were prepared from frozen/thawed 100 g pieces of diaphragm muscle and tested for the presence of BFV-specific antibodies. ELISA test with recombinant Gag protein was adopted, validated and used as described by Romen et all. 2007. Whole genomic DNA was extracted from meat juice samples as well from all organs of 17 seropositive cows. BFV DNA was detected by nested PCR and qPCR, as was described by Materniak et all. 2010.

Specific antibodies were detected in 26 meat juice samples and 100% of them showed concordant results when sera collected from the same animals were tested. Application of nested-PCR showed the presence of BFV DNA in most of the organs of all animals. The highest viral load was detected in liver, lungs and spleen, as compared to the peripheral blood. Our results show that meat juice can be easily and successfully used as sample of choice for detection of BFV specific antibodies by ELISA in slaughtered cows in the absence of serum samples. Furthermore, nested PCR and qPCR can be also applied for direct detection of BFV when muscles or other tissues are examined.

D 11: DETECTION OF NAIROBI SHEEP DISEASE AND RIFT VALLEY FEVER VIRUSES GENOMES BY REAL-TIME POLYMERASE CHAIN REACTION

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Key words: Real -Time PCR

Nairobi sheep disease virus and Rift Valley fever (hereinafter NSD and RVF, respectively) (family Bunyaviridae) virus are the etiological agents of acute vector-borne diseases of human, cattle and small ruminants in Eastern and Southern Africa and in Asia. Both viruses belong to the objects of 3 group of pathogenicity for humans and animals, they cause epidemics and epizootics causing enormous damage to the population and agriculture.

Both viruses cause hemorrhagic fever with lesions of the internal organs and the mortality rate ranges from 40 to 90%. In connection with a similar clinical picture of infections caused by these viruses, development of tools for rapid and reliable differentiation of these is urgent. One of these tools is real –time polymerase chain reaction.

Nucleotide sequences of different NSD virus and RVF virus strains, available in Gene Bank database, were analysed and two oligonucleotide primers pairs were selected for amplification of gene encoding nucleocapsid protein N of both viruses.

The detection of amplified products was performed by recording fluorescence, released during fracture of linear hybridization probes technology Taq-man, labeled with fluorophores FAM and Cy5.

Analytical sensitivity rate of Real-Time PCR was determined by examining the samples of RNA isolated from serial tenfold dilutions of cultures of saiga kidney cells infected with NSD virus (strain "MM/K-05") and RVF virus (strain "1974 VNIIVViM"). Detection limit was considered the maximum dilution at which recorded a positive result. The calculated value of analytical sensitivity of RT-PCR for NSD virus was 2,2 lg TCID50/cm3 and for RVF virus -2,5 lg TCID50/cm3.

To determine the specificity of the test system the samples of RNA isolated from suspensions of the brain of white mice, intact and Akabane virus-infected (strain "B8935") were examined. All studied samples were negative for the presence of the NSD virus and RVF virus genomes. In addition, it was shown that the primers and probe complementary to the genome of the NSD virus, did not interact with the genome of the RVF virus, and vice versa.

Thus, the test –system for detecting disease virus genomes Nairobi and Rift Valley fever bassd on the multiplex RT-PCR was designed. The test –system allows to detect the genomes of NSD and RVF viruses, and simultaneously differentiate them from each other and other members of the family Bunyaviridae.

D 12: DETECTION OF NEW/RE-EMERGING VIRUSES USING THE EPIZONE BIOCHIP 5.1 MICROARRAY

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VLA^1 ; CVI^2

Key words: New/re-emerging viruses, Biochip 5.1 virus-microarray, EPIZONE WP 4.2

Introduction

The BioChip microarray was initially adapted from DeRisi Viro3 chip. It has since been updated several times, first, as part of the UK BioChip project and subsequently, as part of Epizone WP 4.2. The updates were to cover probes of the DeRisi's Viro4 chip, but more recently also to include completely new probes to viral entries in GenBank not included before. The current version of BioChip is 5.1 and encompasses 15K 60-mer probes which are synthesized in situ in an 8 x 15K format. The chip covers about 2000 virus species; each represented by 2 to 20 probes. According to the manufacturing process, we have a significantly increased flexibility to adopt the probes, which enables us to carry out an annual update. In addition to adding probes to new viruses described, we have particularly expanded the number of probes against animal viruses. Through this, we increased the depth of coverage considerably, thus increasing the likelihood to detect viruses related, but not identical to those described already.

Methods

Methods to isolate viral RNA and DNA sequences from different types of clinical samples (serum, plasma, trachea, mouth swaps, lung lavages, spinal cord, faeces) were optimized after pre-treatment with RNAse and DNase, or no pre-treatment. All samples are routinely spiked with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Circovirus (PCV2), and randomly amplified to a PCR-Ct value (19-20) that facilitates detection of PRRSV and PCV2 probes with an intensity well above background. Random amplification reactions with Ct values of \leq 19-20, prepared from field sera of PRRSV and PCV2 infected pigs, scored positive for these viruses on EPIZONE arrays. Detection of spiked PRRSV and PCV2 in tissue samples with the array often failed due to the high level of host RNA and DNA present in these samples. Currently, methods are developed to reduce the level of host RNA and DNA in tissue samples, and to identify viral probes which significantly hybridize with host sequences.

Results

Hybridisation of the EPIZONE arrays with randomly amplified targets form clinical samples of farm and wildlife animals suffering from diseases with an unknown cause detected several existing and new/re-emerging viruses.

CVI (The Netherlands)

In swaps of pigs suffering from airway problems several Swine Influenza Viruses were detected.

In mouth swaps and plasma samples of calves with mouth blisters and fever, Bovine Kobuvirus and the rodent retrovirus "Intracisternal A-particles" was detected. Results of these hybridisation experiments were confirmed by virus-specific PCR and/or virus isolation.

VLA (United Kingdom)

A sapelovirus (formerly PEV-8 was detected in association with encephalitis in UK pigs. Furthermore, a teschovirus (PTV-5) was detected from the faeces of a healthy pig in Humberside, UK. An international investigation into encephalitic disease in horses in Israel resulted in the detection on the BioChip of an orbivirus, equine encephalosis virus; this is the first report of this virus outside southern Africa (Mildenberg et al., Transbound Emerg Dis. 2009 Oct;56(8):291). A new species of rotavirus was discovered in red squirrels with intestinal intussuception (Everest et al., Vet Rec. 2009 Oct 10;165(15):450), proving the use of microarray in investigation of wildlife diseases.

D 13: DETECTION OF TYPE 1 OSTREID HERPES VARIANT (OsHV-1 μvar) WITH NO ASSOCIATED MORTALITY IN FRENCH-ORIGIN PACIFIC CUPPED OYSTER CRASSOSTREA GIGAS FARMED IN ITALY

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Key words: Herpes virus, Oysters, Italy, Diagnosis

Since 2008, the European aquaculture industry has experienced high mortality rates of up to 80% in the Pacific cupped oyster Crassostrea gigas. Between 20 to 100% of breeding Pacific cupped oysters in some French farms and hatcheries have been affected while more recently significant increases in mortality have also been reported in Ireland, the UK and New Zealand. This high mortality in C. gigas has been shown to be associated with a new genotype of the Type 1 Ostreid Herpes virus (OsHV-1), referred to as OsHV-1 microvar. Given the economic importance of oyster aquaculture in many European countries, the EU Commission Regulation n. 175/2010 was enacted in March 2010 to identify the presence of OsHV-1 microvar associated with mortality in oysters in order to reduce the spread of the virus to uninfected regions. According to the regulation, disease control measures must be implemented which include the establishment of containment areas and the restriction of movement from these areas if OsHV-1 microvar and accompanying mortality is identified.

In April 2010, sampling of juvenile Pacific cupped oysters originating from France was undertaken from a farm located off the coast of the Marche region in Italy. Samples were sent to the national reference laboratory for mollusc diseases for bacteriological, histological, electron microscopical and molecular analysis. Classical and Real-Time PCR indicated the presence of the microvariant OsHV-1 microvar despite the absence of clinical and pathological signs normally associated with the presence of this variant. Further molecular and sequencing analysis revealed the presence of OsHV-1 microvar and OsHV-1 DNA in one oyster indicating that simultaneous infection by both viruses can occur. This is the first report of the presence of OsHV-1 microvar has been provided. Due to this growing interest in the oyster farming industry in Italy together with the results obtained from this study and the increasing number of countries reporting OsHV-1 microvar associated mortality, it is of importance that surveillance and monitoring programs for diseases of molluscs are implemented both at a national level and on internationally traded molluscs.

D 14: DEVELOPMENT OF A DOUBLE RECOGNITION IMMUNOASSAY FOR DETECTION OF ANTIBODIES SPECIFIC OF RIFT VALLEY FEVER VIRUS

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INGENASA¹; CISA-INIA²

Key words: Diagnostic, RVFV, DR assays, ELISA

Introduction and Objectives

Rift Vallet Fever (RVF) is a peracute or acute zoonotic disease of domestic ruminants in Africa. It is caused by single serotypes of a mosquito-borne Bunyavirus of the genus Phlebovirus. The disease occurs in climatic conditions favouring the breeding of mosquito vectors and is characterized by liver damage. The disease is most severe in sheep, goats and cattle, in which produces abortions in pregnant animals and high mortality rate in newborn, but there are other animal's species susceptible like camels or buffalo. Humans are susceptible to infection through contact with infected material or mosquito bites. Infection of humans by vectors is a striking feature in countries with a relative small population of animal host. In such areas, RVF may be recognized first in humans. RVFV has caused serious human infection in laboratory workers. Staff should either be vaccinated and work under containment level (3-4), or wear respiratory protection when working with infected animals or when performing post-mortem examinations. Due to RVF has been catalogued by OMS as a re-emerging disease, it is very important to employ preventive measures in order to control it at very early stages. INGENASA, in collaboration with CISA-INIA has developed a precocious, sensitive and highly specific new immunoenzimatic assay able to detect antibodies specific of protein N.

Materials and methods

The new assay is based on the patented double recognition enzyme linked immunoassay technique using RVFV N recombinant protein both as conjugate and for coating. For the evaluation of the assay, different sets of samples have being used:

• 4 sheep experimentally vaccinated and challenged which were bled at different days post vaccination and post challenge.

- 25 camels positive by seroneutralization (SN).
- 490 sheep, 94 cattle and 141 camels from free zones.

Results

Statistical study of the results was made by ROC analysis and distribution curves of the OD values obtained, using Medcalc® software. The distribution curve showed the separation between positive and negative populations and the ROC analysis allowed determining the sensitivity and specificity respect of different cut offs.

This study showed 99.5% specificity using sera from free areas. Analytical sensitivity indicates that the assay is able to detect specific antibodies since day 10 post vaccination and before day 14 post challenge. 92% of camels positive by SN were detected positive by this assay and visible correspondence between SN titre and DR OD has been observed.

Discussion and conclusions

These preliminary results have indicated that the Double Recognition Immunoassay developed by INGENASA has a specificity of over 99% and detects specific antibodies to RVFV at very early stages of infection. Further studies are being prepared in order to confirm analytical and diagnostic sensitivity.

Its capacity for early detection of antibodies, its possibility of multispecie use and its ability for detection of all kind of immnuoglbulines announce the assay as a useful tool for surveillance of negative herds and to control the serological status of new animals.

D 15: DEVELOPMENT OF A ONE-STEP SYBR GREEN-BASED REVERSE TRANSCRIPTASE-REAL-TIME PCR FOR DETECTION OF FLAVIVIRUSES IN POOLED MOSQUITOES

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$IZS-Ve^1$

Key words: Mosquito-borne diseases, Flavivirus, diagnostic, screening

Entomological monitoring is an important part of the vector-borne diseases surveillance systems, provided that rapid and sensitive techniques are available for detection of pathogens in arthropod vectors. Several PCRs are available in literature for Flaviviruses detection in biological samples or vectors, although not validated in pooled mosquitoes. In this study, a novel one-step SYBR Green-Based Reverse Transcriptase-Polymerase Chain Reaction assay (RRT-PCR) was developed for the universal detection and identification of Flaviviruses. The sensitivity of the method was assessed for West Nile virus (WNV) in pooled mosquito samples in laboratory and then its performance verified in the field during the 2010 entomological surveillance for WNV in north-eastern Italy.

Materials and Methods

The used primers (MAMD and cFD2; Scaramozzino et al., 2001) are specific for the conserved region of the non-structural NS5 gene and flank a product of 252 bp (location 9006- 9258) which enable the detection of several Flaviviruses including Yellow fever, Dengue (DEN-1,-2,-3, and -4), Tick-borne encephalitis, Japanese encephalitis, St. Louis encephalitis, Usutu virus (USUV) and WNV. The sensitivity of the RRT-PCR was determined by a 8-fold serial dilution (from 105 to 10-2 TCID50/ml-1) of inactivated WNV (kindly supplied by the Department of Microbiology and Infectious Diseases, Faculty of Veterinary Science, Szent István University, Hungary) spiked in laboratory reared pathogen free pooled mosquitoes (50 specimens) of the species Culex pipiens. RNA extraction from pooled mosquitoes was confirmed with an in-house one-step SYBR Green RRT-PCR using the primers EUKF and EUKR (Lopez-Andreo et al., 2005). For intra-assay variability, each dilution was analyzed in triplicate. Samples with two or more positive reactions were scored as PCR positives. For inter-assay variability, each dilution was analyzed in 3 different runs performed by two distinct operators on different days. The results were confirmed by direct amplicons sequencing and by sequencing of the products of a universal JEV group specific RT-PCR (Bakonyi et al., 2006).

During summer 2010, 2951 pools corresponding to 126957 mosquitoes were examined using this technique.

Results

The SYBR Green-Based RRT-PCR showed a sensitivity for WNV of 102 TCID50 in 50 pooled mosquitoes. During the 2010 surveillance campaign for WNV in Veneto region (north-eastern Italy), the technique allowed the detection of WNV in 10 Cx.pipiens pools and of USUV in other 23 pools. Results were confirmed by sequencing and by the National Reference Centre (CESME, IZS Teramo). In addition, 19 pools of other mosquito species (Aedes vexans and Ochlerotatus caspius) were positives for Mosquito Flavivirus.

Conclusions

RRT-PCRs using SYBR Green have several advantages, allowing to process a large number of samples more rapidly than conventional PCRs, being less prone to contamination and permitting direct sequencing of the amplification products. The availability of new tools for the rapid detection of Flaviviruses in vectors is of great importance for the surveillance of mosquito-borne diseases, in order to immediately apply measures to prevent veterinary and humans cases.

Acknowledgement: This work was supported by the Public Health and Screening Service, Veneto Region and by the Italian Ministry of Health

D 16: DEVELOPMENT OF REAL-TIME PCR METHOD FOR THE DETECTION OF SIBERIAN STURGEON HERPESVIRUS

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Key words: Real-Time, PCR, Siberian sturgeon herpesvirus

Herpesviruses form one of the largest group of viruses causing economically significant diseases in fish. The pioneer study of viral diseases of sturgeons have been conducted by professor Ronald Hedrick (USA), in whose laboratory several cell lines of the white sturgeon (Acipenser transmontanus) were established and first sturgeon viruses were isolated. To date, around 10 different viruses have been found in the North American sturgeons, of which two agents belong to the family Alloherpesviridae order Herpesvirales. A herpesvirus was also isolated from shortnose sturgeon (A. brevirostrum) in Canada, and in 2006 from the Siberian sturgeon (A. baeri) in Russia. The latter one is believed to cause about 50% mortality in cultured juvenile sturgeon.

Rapid diagnostic methods for sturgeon herpesvirus have not been developed, and the traditional methods are labor-intensive, lengthy and do not always meet the requirements of modern aquaculture. The aim of the present study was to develop a real-time PCR based method for detection of Siberian sturgeon herpesvirus and to evaluate the applicability of its use on cell culture grown virus and fish clinical samples.

Samples of heart, liver, kidney, spleen, posterior intestine, swim bladder, mouth, fins and skin mucus were collected from experimentally infected fingerling sterlet (A. rhutenus), and naturally infected Siberian sturgeon yearlings.

Primers and probe used in the real-time PCR: forward primer - gCAACAAggCTCggTTAgATg; reverse primer - gCgTCgAggAATTgTTTCTgg; probe - [FAM]-TgTgTTggCggTTggCAACTTACA-[BHQ1]. Selection of primers and an oligonucleotide probe was carried out using the program Oligo 4.0 on the basis of the nucleotide sequence in GenBank [NCBI GI: 288975339]. The real-time PCR was performed on the RotorGene-6000 cycler («Corbett Research», Australia), and the data obtained were analyzed using the software provided with the machine.

The specificity of this method was confirmed by sequencing the PCR amplicons. In parallel, virus titers in the clinical samples were determined on the cell line SSO-2 (Siberian sturgeon organs).

The overall results of destiny the developed real-time PCR were as follows:

1) The genome of Siberian sturgeon herpesvirus was detected by real-time PCR both in infected cell culture and fish clinical samples;

2) By both methods (cell culture and real-time PCR) the virus was revealed during the acute stage as well as at the final stage of the epizootics;

3) The diagnostic sensitivity of virus detection by real-time PCR was lower than virus isolation during the acute phase of disease, but surpassed it on final stage of disease. The primers used were specific to the Siberian sturgeon herpesvirus and did not produced false-positive results with DNA isolated from cell cultures, clinical samples of healthy sturgeons or heterologous viruses.

The work on optimization of developed real-time PCR is being continued.

D 17: DIAGNOSIS OF AN OUTBREAK OF SHEEP POX ASSOCIATED WITH GOATPOX VIRUS

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 $LVRI^1$

Key words: Goatpox virus, sheep, infection, p32 gene

A CPV/GSgulang/09 strain of capripox virus was isolated by inoculating samples into Vero cells from lung tissues of sheep (Small-tailed Han sheep)located in Gulang city of Gansu province, whihe suffered from pox disease. PCR-RFLP and sequence analysis of p32 gene was conducted for classification of this virus. Two bands which are specific to goatpox virus (GPV) were observed after the product of p32 gene amplified from genomic DNA of CPV/GSgulang/09 was digested with the endonuclease of Hinf □. Three nucleotide bases for coding residue of aspartic acid which are located at 166-168 positions of p32 gene sequences from different capripox virus available in GenBank showed that CPV/GSgulang/09 shared the homology of 98.1% -98.3% with SPV and 99.2%-99.9% with GPV, respectively. In the phylogenetic analysis, CPV/GSgulang/09 was more closely related to GPV strains and clustered into the GPV sub-branch of phylogenetic tree constructed based on p32 gene. Results showed that an outbreak of sheep pox associated with GPV was occurred for the first time in China.

D 18: DOUBLE RECOGNITION DIRECT IMMUNOASSAYS FOR DETECTION AND DIFFERENTIATION OF ORBIVIRUS VP7 SPECIFIC ANTIBODIES IN SERUM

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INGENASA¹

Key words: Orbivirus, Diagnostic, AHSV, EHDV, BTV

Introduction and Objectives

The recent changes in Bluetongue situation have promoted the idea of the possibility of similar spreading of other orbivirus such as AHSV and EHDV.

Due to their pathogenesis and high transmission / diffusion ability, these diseases are included in the OIE list so every outbreak in a country implies serious restrictions to trade causing major economic loss.

INGENASA had already designed in 2007 an immunoassay (INGEZIM BTV DR) based on the patented double recognition technique for detection of antibodies specific of BTV VP7 protein. Anticipating a possible similar spread of these other two diseases, new immunoassays based on the double recognition technique for detection of AHSV and EHDV VP7 specific antibodies, are being developed.

Materials and Methods

The three assays use VP7 recombinant proteins as conjugate and for coating.

Different sets of samples were used for evaluation of the different kits:

• Reference Sera: 7 goat and 9 Guinea Pigs infected with different serotypes of AHSV; 24 calf infected with different BTV serotypes and sera positive to 8 different EHDV serotypes.

• Experimental sera: 8 sheep infected with different serotypes of AHSV; 2 horses infected with serotypes

4 and 8 respectively, sera from BTV serotypes 4 and 8 experimentally infected animals (cattle and sheep. • Positive field sera: 116 horses positive to AHSV, 288 sera previously characterized as positive to BTV by other techniques, 7 sera positive to EHDV from Morocco.

• Negative field sera: 743 AHSV negative horses, 758 sera from BTV free herds, 250 EHDV negative deer from free areas.

• 714 sera of new world camelids (llama, alpaca)

Results

INGEZIM BTV DR showed 100% and 99.9% diagnostic sensitivity and specificity respectively. All Reference Sera specific to the 24 BTV serotypes were positive. The assay was able to detect specific antibodies in experimental sera as early as at 5 days p.i. in infected sheep and 7 days p.i. in infected cattle.

The ELISA developed for AHSV detected specific antibodies at day 7 p.i. and all Reference Sera specific to the 9 AHSV serotypes. Diagnostic specificity was 99% and diagnostic sensitivity 100%. ELISA DR for EHDV detected all Reference Sera specific to the serotypes described. All the sera checked so far from free areas were negative. EHDV specific antibodies were detected in the 7 positive sera from Morocco.

Each of these assays only detects the specific antibodies at which it is targeted.

Discussion and Conclusions

The results obtained have indicated that the assays for BTV and AHSV are useful tools for disease surveillance and control because of their characteristic sensitivity and specificity (nearly 100%) and their capacity for early detection of antibodies. The fact that none of them showed any crossreaction with specific antibodies different from the one at which they have been targeted allows to clearly identify the disease in those cases in which different orbivirus could affect the same species.

Further studies will be necessary in order to confirm that EHDV ELISA DR shows a similar performance as the immunoassays for the other two orbiviruses.

Acknowledgements

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D 19: EPIZONE: CYPRINID HERPESVIRUS 3: TO BE, OR NOT TO BE

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Key words: carp; diagnostics; koi herpesvirus; CyHV-3

Cyprinid herpesvirus 3 (CyHV-3), also known as koi herpesvirus (KHV), is a devastating virus of common carp and ornamental koi. With the international trade in koi and carp the virus has spread rapidly over the globe to major carp culture areas in the last decade. The disease has been listed by both the OIE and the EU. Currently detection of CyHV-3 takes place by PCR-techniques as the sensitivity of the available cell lines for virus isolation is too low. So far the complete genome of three CyHV-3 isolates from different geographical locations has been sequenced showing very limited differences in the genome sequences of these three isolates.

Cyprinid herpesvirus 3 is member of the genus Cyprinivirus to which also CyHV-1 (carp pox herpesvirus) and CyHV-2 (goldfish haemotopoietic necrosis virus) belong. During routine diagnostic testing for CyHV-3 by PCR, CyHV strains were detected by serendipity which were very closely related but yet distinct from CyHV-3. Up to now isolation of this CyHV-3 variant using cell culture has not been successful. Sequences of the CyHV-3 variant were obtained for the polymerase gene and major capsid protein gene using general cyprinid herpesvirus primers (developed by CEFAS). The CyHV-3 variant has approximately 96% identity to the polymerase gene and 97% identity to the major capsid protein gene. The koi bearing the CyHV-3 variant virus were clinically healthy and originated from areas with no actual CyHV-3 outbreaks. It is therefore difficult to assess the implication of this CyHV-3 variant to the carp and the diagnostics of CyHV-3. The isolates might represent a low- or non-pathogenic variant of CyHV-3 and it might be questioned whether this CyHV-3 variant should be notified or not or be subjected to control measures.

D 20: EPIZONE: DIAGNOSTICS THEME, RESEARCH IN WORK PACKAGE 4.4: PEN-SIDE TESTS

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Key words: diagnostics, pen-side, work package 4.4

The purpose of work package 4.4 is to investigate methods for the detection of pathogens in the field or modestly equipped/temporary laboratories. The research in WP 4.4 focuses on detection methods for genomic material or proteins from the pathogens. In genomic testing, the most complete systems designed for pen-side use automatically process raw sample into a diagnostic result. They are also the most advanced and work with these has involved two commercial collaborators. This sophisticated approach is the simplest from a user stand point but the most elaborate to develop and expensive to use. The most basic methods involve using isothermal PCR chemistry so that only a simple heating block or water bath is needed; extraction can be performed using filter paper or an LFD or not at all (for ex. use whole or diluted blood) and samples are detected using intercalating dyes or on an LFD. In between, various combinations are being studied that involve use of more sophisticated but portable equipment such portable real-time PCR instruments and isothermal devices but often with more simplified sample preparation. In assays focusing on protein detection, three antigen lateral flow devices (LFDs) have been produced: a pan-FMDV, a FMDV SAT2 specific, and an SVDV LFD. The technology used allows for detection in the field and has an analytical sensitivity comparable to antigen ELISA. Target viruses in WP 4.4 have included foot and mouth disease virus, swine vesicular disease virus, lyssavirus, African swine fever virus, influenza virus and peste-des-petits-ruminants virus. The objective has been to produce and identify the most suitable methods for pen-side testing. For more information, please visit the poster presented at the Epizone 5th Annual Meeting, April 11-14, 2011, in Arnhem, The Netherlands.

D 21: EPIZONE: EXPERIMENTAL INFECTIONS OF FALCONS WITH WEST NILE VIRUS LINEAGE 1 AND 2

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Key words: West Nile virus, experimental infection, falcons, lineage 1 and 2

West Nile virus (WNV) is a flavivirus that is transmitted by blood-suckling mosquitoes. WNV is an important zoonotic virus that uses birds as primary vertebrate reservoir hosts (enzootic cycle). Some bird species like ravens, falcons and jays are highly susceptible and develop deadly encephalitis while others undergo only a subclinical infection. A wide range of mammals (infections of humans can cause encephalitis with fatal outcome) and reptiles are also susceptible and can develop fatal diseases.

Furthermore a high mortality in raptors (hawks, falcons and other birds of prey) associated with WNV infections lineage 1 was described in the USA. Besides the USA, WNV outbreaks in humans and animals were recently reported in the Mediterranean region. Associated with this, a high number of birds, e.g. pigeons, crows and magpies tested positive for WNV, but no increased number of deaths occurred among feral birds related to these infections. When the virus emerged in Spain in 2007 it also affected two Imperial Eagles. In the last years the presence of a lineage 2 WNV infection in birds of prey in Hungary and Austria was also diagnosed which was able to infect hawks and falcons and also parrots with fatal outbreaks. These findings indicate a high susceptibility of raptors for both WNV lineages (WNV lineage 1 and the Austrian/Hungarian WNV lineage 2 isolate).

The aim of this experimental study was to evaluate the effect of WNV lineage 1 (NY99) and 2 (strain Austria) using three different virus doses in falcons to clarify the role of these species in WNV enzootic cycle.

For the infectious trial we used for each virus lineage six captive-bred hybrid falcons (Falco rusticolus x Falco cherrug). Always two falcons were subcutaneously inoculated with low, intermediate or high dose of WNV-NY99 or WNV-strain Austria. Blood samples, cloacal and oropharyngeal swabs were collected at 2, 4, 6, 10 days post infectionem (dpi) and at the end of the experiment after 2 or 3 weeks. Clinical signs were observed over time and birds were necropsied between 14 and 16 dpi (lineage 1) or 20 and 21 dpi (lineage 2). All experiments were carried out under biosafety level 3 conditions.

Following the challenge with virus from both lineages falcons receiving high or intermediate doses developed clinical signs and typical gross pathological findings were observed. Detailed results of virus re-isolation, qRT-PCR and serology will be presented for a range of tissues and bodily fluids.

D 22: EPIZONE: RING TRIAL ON AFRICAN SWINE FEVER VIRUS (ASFV) REAL-TIME PCR

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Key words: African swine fever, real-time PCR, ring trial, diagnostics

As a part of the EPIZONE WP4.1: Real-time PCR diagnostics, a ring trial on real-time PCR for the detection of African Swine Fever Virus (ASFV) DNA was carried out.

The ring trial comprised a collection of 27 blind DNA samples to be tested by ASFV real-time PCR methods established at each participating laboratory, as well as by one common reference method. The aim of this study was the identification of useful ASFV real-time PCR assays, in terms of sensitivity, specificity, repeatability, and reproducibility.

The ring trial was organized by the European Union Reference Laboratory (URL) for African Swine Fever (ASF), CISA-INIA, Valdeolmos, Spain, with assistance from the other ASF core group members within the EPIZONE WP4.1: DTU, Lindholm, Denmark, and SVA, Uppsala, Sweden. A total of 12 laboratories took part in the ring trial, representing nine ASF National Reference Laboratories of EU member countries and some other EPIZONE partners working on ASF.

Clinical material collected from experimentally ASFV infected pigs or ASF viral suspensions were diluted in healthy donor pig material in order to prepare the sample panel. The coded DNA collection finally comprised 23 porcine serum, blood and tissue samples containing different amounts of reference ASFV isolates representative of genotypes I, II, IX, and X, and 4 ASFV negative samples. A primer/probe mix containing both primer set and TaqMan probe described by King et al (2003), and the URL SOP based on the aforementioned method (considered as the reference assay in this study) were provided also to the participants.

All 12 participants performed the real-time PCR recommended in the OIE Manual (described by King et al, 2003), either following the provided URL SOP (8x), other modified in-house procedures (2x) or both (2x). One lab ran a modified procedure of the PCR method described by Zsak et al, 2005. Two labs tested the only currently commercially available ASFV PCR kit (Tetracore Inc., Rockville, MD, US), which includes all reagents dried down, as well as a rehydration buffer and a positive control. Seven additional in-house-developed real-time PCR methods were carried out by eight participating labs, though all unpublished so far. It is noteworthy that these new techniques are based on different molecular approaches: FRET probes, ASF/CSF triplex PCR, conventional TaqMan probe, PriProET system, LATE-PCR technology, SybrGreen, and UPL commercial probes. In addition, it is remarkable that all the PCR methods reported in the ring trial are based on the amplification of a DNA fragment within the VP72 ASFV genome region.

All PCR systems worked rather well and were capable of detecting the range of viral p72 genotypes considered in the study. However, the methods recently developed generally increased the sensitivity and repeatability of the King-based PCR assays. Furthermore, the commercial ASFV PCR kit also demonstrated a fine sensitivity in the different platforms employed.

In conclusion, a range of valuable molecular tools are currently in use for the ASFV detection, including one commercial kit, although most of them are not yet published.

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D 23: GENETIC DIAGNOSTICS OF OVINE PULMONARY ADENOMATOSIS

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Key words: PCR, retrovirus,LTR region, env-gene

Adenomatosis (also called adenomatosis, ovine pulmonary adenomatosis, Jaagsiedte, bronchoalveolar lung carcinoma or bronchoalveolar cancer) is a slowly developing contagious infection characterized by long incubation period, prolonged course and progressive growth of alveolar and bronchial epithelium accompanied by formation of tumours. Currently ovine adenomatosis is registered in all continents. In ovine genome some endogenous retrovirus sequences similar to nucleotide sequences of ovine pulmonary adenomatosis virus genome have been found.

In the course of a real time PCR-based test system development some specific primers complementary to LTR region were selected to be used for identification of the exogenous ovine adenomatosis virus. To identify the endogenous ovine pulmonary adenomatosis virus in a standard PCR analysis the primers complimentary to the 3'-terminus of ENV gene and a U3-region of LTR were used.

For the investigations some affected animals with pronounced clinical signs (like coughing, dyspnea and/or abundant nasal discharge) were selected. As negative controls the blood and lungs of clinically healthy sheep and goats were used.

In the analyses of sheep/goat blood samples only the DNAs of endogenous type adenomatosis virus were found, the virus DNAs being isolated both in sheep and goat. To determine the presence of endogenous ovine adenomatosis virus in other animal species, blood samples from cattle, rabbits and pigs were analysed. The obtained data suggested that the endogenous ovine adenomatosis retrovirus was present in sheep and goats only.

In the analysis of lung suspensions from sheep with clinical and pathological signs of ovine adenomatosis the both virus types were detected.

The test system using real time PCR which we have developed was applied in diagnostic and monitoring research works aimed at adenomatosis-affected sheep detection. We examined some 582 blood & lung samples from sheep and goat, the 7 ovine samples among them being positive for ovine pulmonary adenomatosis. The positive results were confirmed histologically.

The comparative analysis of the obtained results showed that the site of the endogenous virus we have amplified had 100% homology while the exogenous virus had 97% homology with the isolates in GenBank.

Thus, we have demonstrated that the PCR approach can be used to detect ovine pulmonary adenomatosis virus genome.

D 24: INCREASING SENSITIVITY OF IBR ELISA TESTS FOR BULK MILK BY SAMPLE TREATMENT

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Key words: IBR, BHV1, bulk milk, sensitivity increase

Infectious bovine rhinotracheitis (IBR) control programs are in place in several European countries. Diagnosis of IBR is mostly based on BHV1 antibody detection using ELISA tests. For testing serum samples, the IBRgB blocking ELISA is considered to have the highest sensitivity. The indirect IBR ELISA performs best in milk samples, while IBRgB and IBRgE blocking ELISAs show sensitivity problems in pooled and even in individual milk samples.

This has an impact on efficiency and costs of IBR control programs. In Germany 85,1% of dairy and suckler cow herds are free of IBR or IBRgE. But in 2008 3.68 million blood and individual milk samples were tested compared to just 281.442 bulk milk samples. IBRgE marker vaccinated herds are tested on individual blood samples since the IBRgE ELISA is not recommended in Germany for testing bulk milk samples. Only non-vaccinated herds can participate in cost-efficient bulk milk testing in Germany. Problems arise when marker vaccinated cows contribute to bulk milk which is tested by indirect IBR-ELISA, since this is causing false positive reactions.

Increasing sensitivity of bulk milk ELISAs by milk concentration was performed for years for Brucellosis, Leucosis and IBR testing but the procedure described previously was tedious and not well standardised.

Labor Diagnostik Leipzig developed a new method for the concentration and purification of antibodies from milk and bulk milk samples. Compared to previous methods CATTLETYPE® Milk Prep Kit is a ready-to-use set of reagents and allows an easy, standardised and user-friendly sample-prep-procedure. Antibodies from 50 ml bulk or pooled milk bind to a special matrix. As a result of the procedure, 200 µl concentrated and purified milk antibodies can be assayed.

In order to evaluate the performance of CATTLETYPE® Milk Prep Kit, reference milk samples from Friedrich-Loeffler-Institute in Germany and international ring trial samples were tested using IBRgB and IBRgE ELISA. Furthermore milk samples from a herd with a history of being IBR positive for many years (herd A) and from one herd with a recent BHV1 re-infection (herd B) were tested individually, in pools of 1 positive in 49 negative milk samples, and those milk pools after sample concentration using CATTLETYPE® Milk Prep Kit.

2 of 37 (5.4%) IBRgE antibody positive milk samples of the herd A and 3 of 84 (3,6%) of herd B were detected positive in a pool of 50. After concentration of those milk pools, 28 of 37 (75.7%) and 61 of 84 (72,6%) samples scored positive. Field and reference milk samples with strong and medium strong signal in IBRgE ELISA are mostly detected in pools of 50 after the sample prep procedure. In the German state of Thuringia a pilot project was initiated were IBRgE marker vaccinated herds free of IBR and marker vaccinated herds with IBRgE positive cattle are currently tested from serum and bulk milk after sample concentration for IBRgE in parallel. Results will be presented.

The presented method of concentration and purification of antibodies from pooled and bulk milk samples with CATTLETYPE® Milk Prep Kit increases sensitivity of IBRgE and IBRgB blocking ELISA tests. It will help to do cost-efficient bulk milk testing in IBR marker vaccinated cattle herds with a high sensitivity, monitoring free herds for introduction of IBR. Since the sample prep protocol is not assay specific, the procedure has some possible other applications and can improve sensitivity of other bulk milk based disease surveillance programmes.

D 25: MAPPING OF NEUTRALIZING SITES TARGET OF TWO MONOCLONAL ANTIBODIES SPECIFIC FOR E PROTEIN OF WEST NILE VIRUS

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IZSLER¹

Key words: West Nile virus, escape mutants, monoclonal antibodies.

Introduction

West Nile virus (WNV) is a single-stranded RNA virus member of the Japanese encephalitis virus serocomplex in the genus Flavivirus. The WNV genome encodes three structural proteins (C, prM/M, and E) and seven non structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). The envelope glycoprotein E of WNV folds into three domains DI, DII, DIII and is responsible for virus attachment and entry into cells. In particular DIII is the putative receptor-binding domain and an important target for specific neutralizing antibodies and in vivo protection. The purpose of this work was to characterize two monoclonal antibodies (MAbs), 3B2 and 4D3, previously produced and representative of two different WNV specific and neutralizing epitopes (1), through selection and sequence analysis of MAbs escape mutants.

Methods

Antigenic variants were selected by growing WNV E101 in Vero cells in the presence of neutralizing MAbs 3B2 and 4D3. Serial 10-fold dilutions of the virus were incubated with MAbs at high concentration prior to infection of Vero monolayer with the virus-MAb mixture. Samples of the supernatants were taken 7 days post-infection and screened for the presence of escape mutants by sandwich ELISAs performed using the non neutralizing MAb 2A8 cross-reactive with flaviviruses as catcher and MAbs 3B2, 4D3 and 2A8 in parallel as conjugates. Mutants that were not recognised by 3B2 and/or 4D3 MAbs while maintaining reactivity with 2A8 were selected and amplified in Vero cells and their capability of escape from MAbs neutralization were investigated by virusneutralization tests performed using the selected WNV variants and MAbs 3B2 and 4D3 in Vero cells.

Viral RNA was extracted from wild-type WNV E101, WNV 3B2 and 4D3 escape mutants (EM3B2, EM4D3) and the complete region of E gene was amplified by RT-PCR and sequenced.

Results

One neutralization-resistant escape mutant was identified for each MAb 3B2 and 4D3 and purified by at least three cycles of endpoint dilution in the presence of the selecting MAb. Virusneutralization assays showed that neutralizing MAb 3B2 was unable to neutralize EM3B2 but retained strong neutralizing activity against the EM4D3, while MAb 4D3 was unable to neutralize EM4D3 but retained strong neutralizing activity against the EM3B2. Comparative sequence analysis of selected escape mutants and wild-type WNV E101 showed a single-nucleotide mutation in the E protein gene of EM3B2 at residues 919 (AAG \rightarrow GAG) encoding an amino acid change at E307 (Lys \rightarrow Glu). A different single-nucleotide mutation was identified in the E protein gene of EM4D3 at residue 827 (AGC \rightarrow ATC) encoding an amino acid change at E276 (Ser \rightarrow IIe).

Conclusions

The most potent neutralizing MAbs to WNV described to date bind to DIII although neutralizing antibodies against other domains have also been described (2,3). Based on the sequence of in vitro neutralization escape variants, MAb 3B2 described here maps to a region on the lateral surface of DIII which has been previously described as an important specific neutralizing epitope for WNV (4,5,6), while MAb 4D3 appears to recognize a novel strong neutralizing epitope on DII that has not been described before with WNV MAbs.

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D 26: MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF VP1 OF PORCINE TESCHOVIRUS ISOLATES IN SPAIN

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CISA-INIA¹; Laboratorio Central de Veterinaria²

Key words: Porcine Teschovirus; RT-PCR; phylogenetic analysis

Introduction

A specific national program for surveillance of swine diseases is being implemented in Spain, which involves feces collection at farms where serologic data raise suspicion of swine vesicular disease (SVD), according to the Manual for Diagnosis of SVD in force in the European Union. In a previous study, approximately 600 fecal samples, representative of main Spain's pig production areas, were examined in 2004-05, and SVD virus was not detected. Instead, other pig enteric viruses were isolated, of which porcine teschoviruses (PTV, a genus within the picornaviridae family) were the most abundant, accounting for 47% of the total number of viral isolates examined (n=206)(1). Here we present the molecular characterization of this group of viruses found in Spain, based on sequencing of the VP1 capsid protein-encoding gene, most frequently used for virus typing in picornaviruses.

Materials and methods

We essentially examined the group of PTV isolates identified in the previous study cited (1), consisting of 97 PTV-positive isolates to which we added 8 more PTV isolates obtained similarly from fecal samples examined later under the same surveillance program. For PTV typing we applied a series of newly developed RT-PCR methods for amplification of the whole VP1-encoding gene, followed by nucleotide sequencing. The sequences obtained were compared to analogous nucleotide sequences available in GenBank. Phylogenetic trees were constructed using the maximum likelihood statistical method.

Results

We obtained 62 complete VP1 sequences out of 105 analyzed. Sequence alignments included 71 reference strains obtained from GenBank for the eleven PTV serotypes known to date. In the phylogenetic tree, the 62 new PTV VP1 sequences obtained in this work separated in twelve clusters, eleven corresponding to each PTV serotype, but one group clustered outside any previously known PTV serotype, within subgroup I, suggesting the existence of an as yet unknown serotype.

The field samples were assigned into clusters as follows: 31 (50%) clustered with PTV-2; 12 (19.4%) with PTV-6; 6 (9.7%) with PTV-4; 3 (4.8%) with PTV-5; 3 (4.8%) formed a new cluster (putative PTV-12); 2 (3.2%) clustered with PTV-7; 2 (3.2%) with PTV-1; 2 (3.2%) with PTV-11, and 1 (1.6%) with PTV-8. Sequences similar to PTV serotypes 3, 9 and 10 were not found within these field samples.

The geographic distribution of these groups could not be associated to particular territories, but rather, co-circulation was found in the same geographic areas.

Discussion and conclusions

The method here described indicates that the molecular analysis for VP1 is a suitable diagnostic tool for the identification of porcine teschoviruses, allowing their classification into the different genotypes described. A recent study performed in Italy, using a similar approach targeting also VP1 of PTV equally suggests the utility of this region for PTV typing (2). An additional PTV genetic cluster has arisen in the present study which might constitute a new PTV serotype. Further studies are in course to clarify this point.

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D 27: MOLECULAR DETECTION OF A MIXED INFECTION OF GOATPOX VIRUS, ORF VIRUS AND MYCOPLASMA CAPRICOLUM SUBSP. CAPRIPNEUMONIA IN GOATS

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LVRI^1

Key words: Goatpox virus, Orf virus, M. capricolum subsp. Capripnemonia, mixed infection, goats

In September 2009, an outbreak of Goat Pox was diagnosed based on clinic signs in a farm which housed 338 local goats in Chongqing of China.The observed clinical signs were ecthyma and accelerated respiration with frequent coughing. Further investigation on etiological agents of the disease in this outbreak was conducted because of a total case fatality rate of 60.2 % in adults(19 of 30) and kids(40 of 68) in this flock. Specific fragments of the p32 gene of Goatpox virus (Gpv), B2L gene of Orf virus (Orfv)and 16s rRNA gene of Mycoplasma capricolum subsp. capripneumonia (Mccp) were synchronously amplified by PCRs from the tissues of 12 dead goats. The PCR products were then cloned, sequenced and aligned with related reference sequences in GenBank for further identification of the pathogens. Results showed that an outbreak of mixed infection of Gpv,Orfv and Mccp was occurred. To the authors' knowledge, this is the first report of mixed infection with Gpv, Orfv and Mccp in goats.This finding is significant epidemiologically and challenges the existing plans for the prevention and control of goat diseases.

D 28: MOLECULAR DETECTION OF FERRIC SIDEROPHORE RECEPTOR GENE IN SWINE ISOLATES OF BORDETELLA BRONCHISEPTICA*

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Key words: Bordetella bronchiseptica, ferric siderophore receptor gene, PCR

Introduction

Bordetella bronchiseptica (Bbr) is a worldwide spread pathogen of pigs' respiratory tract. When infecting the pig alone it may cause mild form of atrophic rhinitis (AR) called NPAR, while invading the respiratory tract in cooperation with Pasteurella multocida (Pm) it may cause progressive changes characteristic for PAR.

Bbr posses different virulence factors, except dermonecrotoxin (DNT) an important factor influencing the pathogenic properties of Bbr is the receptor involved in iron acquisition and transport. This factor is essential for Bbr replication and survival in the environment (1, 2, 3).

The aim of the study was the detection of the exogenous ferric siderophore receptor gene - BfrZ gene in Bbr strains by using three elaborated PCRs.

Material and methods

A total of 182 strains of Bbr isolated from pigs' nasal swabs do not demonstrated typical clinical signs of AR, from 43 farms located in Poland, were tested.

Firstly, swabs were analyzed by standard microbiological methods. Bbr cultures were performed using G20G medium, which contains antibiotics. Plates were incubated at 37oC for 48-72h or even longer.

For detection of BfrZ Bbr molecular techniques such as: classical PCR, Real-Time test PCR with Sybr Green and with TaqMan probe, were elaborated. Primers used in these tests permitted amplification of the 368, 368 and 299bp, respectively. They were designed by ourselves based on sequences from GeneBank. In classic PCR AmpliTaq Gold reagents, while in Real-Time PCRs Qiagen reagents were used.

Results

Seventy three % of Bbr strains tested possessed dermonecrotoxin gene. From all 182 Bbr isolates studied 52.7% gave positive results in all three PCRs used for detection of BfrZ gene. In the case of 31.8% of samples the use of two Real-Time PCRs enabled detection of BfrZ gene, while classic PCR wasn't effective. In case of 9.3% of specimens only Real-Time PCR with TaqMan probe was able to detect BfrZ gene, the other two methods gave negative results. In as little as 2.7% of tested samples BfrZ gene was absent.

Results of our study show that other pathogens causing respiratory diseases in pigs were not detected by elaborated tests which indicate that designed sets of primers were specific only for Bbr. The sensitivity of the elaborated tests were also high and sufficient for diagnostic purpose.

Discussion

Genus Bordetella have wide range of ferric siderophore receptors genes but BfrZ gene, which was used in our study, is present only in Bbr genome so PCR tests for its detection can be used as species specific assay. From all molecular tests used in this study Real-Time with TaqMan probe was the most sensitive. The percentage in the difference in sensitivity between Real-Time with TaqMan probe and with Sybr Green achieved 14.5%, but in comparison to classic PCR the difference increased up to 36.9%. The results obtained in our study shows that primers sets were specific only for DNA BfrZ Bbr and molecular tests can be used to differentiate pigs' strains from those isolated from human. The results shows that many Bbr isolates have ferric siderophore receptor gene what may suggest that its presents increased the level of pathogenicity of mentioned bacteria.

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D 29: NOVEL SEROLOGICAL AND MOLECULAR DIAGNOSTIC TOOLS FOR RVFV DETECTION AND SURVEILLANCE

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Key words: RVFV detection surveillance

Rift Valley fever virus (RVFV) is a mosquito borne pathogen and belongs to the Bunyaviridae family, genus Phlebovirus. This disease causes significant morbidity and mortality in humans and livestock, infects a wide range of vertebrate hosts such as cattle, sheep and goats throughout Africa and is extremely abortigenic. RVFV is considered to have the greatest potential of the possible zoonotic arboviral diseases to spread from Africa to Europe since this virus amplifies productively in numerous of arthropod vectors.

To get more detailed information about the prevalence of this virus in a given area, novel test formats and assays are available, such as a variety of indirect enzyme linked immunosorbent assays (ELISAs) for RVFV antibody detection. Most, if not all, of these ELISAs are based on nucleocapsid protein or crude RVFV antigen.

The aim of the here presented work was to evaluate the sensitivity and specificity of an ELISA based on RVFV structural proteins (G1- and G2-glycoproteins). For this purpose, G1-and G2-glycoproteins were expressed in E. coli, successfully purified and biochemically characterised. Polyclonal rabbit antisera were raised against these recombinant RVFV proteins (nucleocapsid protein, G1 and G2-glycoproteins) as well as a panel of monoclonal antibodies against G1-glycoprotein. Validation of the G1/G2 based ELISAs will be done with positive field sera and defined negative sera in order to define cut-off values and to determine sensitive and specificity of the assay.

For quantification and determination of viral load in blood and tissue samples from RVFV infected animals a novel external calibrator (synthetic RNA control) was created to support quantitative real-time RT-PCR protocols.

In summary, this investigation permits the improvement of seroepidemiological and molecular diagnostic studies on RVFV throughout Africa and Europe for an effective surveillance on RVFV outbreaks and spread in affected countries.

D 30: OPTIMALIZATION AND APPLICATION OF A MULTIPLEX RT-PCR FOR SIMULTANEOUS DETECTION OF ACUTE BEE PARALYSIS VIRUS (ABPV) AND DEFORMED WING VIRUS (DWV) IN POLISH SAMPLES OF THE HONEY BEE (APIS MELLIFERA L.)

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$NVRI^1$

Key words: Multiplex RT-PCR; Honey bee; Detection; Acute bee paralysis virus; Deformed wing virus

The western honey bee, Apis mellifera L., plays a significant role in the global market as well as in Polish economy. Furthermore, by pollinating various plant species honey bees play crucial role in maintaining biodiversity. Nowadays the vast majority of honey bee colonies decline in many countries around the world, still the reason of mortality is unknown. There are few factors that could play a significant role in those serious morbidities. The previous studies suggested that the factors might have cross influence. Honey bee viruses are one of those agents. So far, honey bee has been reported to be the host of at least eighteen viruses. Often an individual insect is infected with more than one virus species at the same time. Among viruses that has been investigated until now, Deformed wing virus and Acute bee paralysis virus are the most common viruses in Polish apiaries and probably have the highest impact on winter bee mortality in Poland. Viral diseases of bees are very difficult to detect and distinguish from each other, because often there are no visible signs of the ailment. Even when the symptoms appear, they are frequently similar to each other and can be observed in a very brief period of time because infected colony die in a short term. Moreover, honey bee viruses easly spread between the individuals as well as the whole colonies.

So far, in our laboratory, a large number of honey bee samples has been investigated using conventional RT-PCR. Each virus species has been detected in separate reaction, which was labor- time- and cost-consuming. For this reason, it is very important to introduce a new, specific and rapid diagnostic method, like a multiplex RT-PCR, which allows simultaneous detection of ABPV and DWV, two important etiological agents. This method would allow to reduce time and costs of research. When it is a single tube reaction, the risk of contamination is reduced. In addition, faster acquisition of result allow beekeepers to faster detain of virus spread. The previous study conducted by other researchers has shown that above mentioned molecular tool is accurate for simultaneous detection of Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV) and Sacbrood virus (SBV).

The goals of this study were optimalization and application of a multiplex reverse transcription-PCR (RT-PCR) for a rapid, specific and sensitive detection of Deformed Wing Virus and Acute Bee Paralysis Virus in Polish bee samples. A few sets of primers, specific for each virus species and a few different kits were tested in a multiplex RT-PCR assay. The individual, conventional RT-PCR assays and the multiplex RT-PCR assay were optimized for highest specificity and sensitivity. The multiplex RT-PCR assay was tested on field samples collected from Polish apiaries. Both DWV and ABPV were detected. Specificity of the PCR products was confirmed by sequencing and a comparison with other confirmed sequences of DWV and ABPV from GenBank database. The best kit for rapid, specific and sensitive multiplex RT-PCR is an accurate tool for rapid, specific and sensitive simultaneous detection of ABPV and DWV in honeybee samples.

D 31: PHYLOGENETIC ANALYSIS OF ROTAVIRUSES FOUND IN TURKEY FARMS IN POLAND

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 $NVRI^1$

Key words: rotaviruses, turkeys, NSP4 gene, phylogenetic analysis

Rotaviruses are a common case of severe, acute gastroenteritis in humans and in various animal species, including birds and mammals. These viruses have a genome of 11 segments of double-stranded RNA and are classified into the Reoviridae family, Sedoreovirinae subfamily. Rotaviruses have been isolated from many avian species as chickens, turkeys, guinea fowls, pheasants, partridges, pigeons and wild birds. Clinical signs include mild to severe diarrhea, dehydratation, poor weight gain, increased mortality however asymptomatic infections have been also observed.

The aim of this study was to determine the prevalence of rotaviruses in commercial meat-type turkey flocks in Poland and provide epidemiological information based on phylogenetic analysis of detected viruses.

During the period between January 2008 – November 2010, clinical samples from 161 flocks (10 individual faecal swabs/flock) from affected and healthy poults, ranging from 7 days to 19 weeks of ages, were collected from different regions of the country. All samples were stored in -200C until processed. After slow thawing each individual swab was hydrated in PBS with antibiotics, incubated for 1 h at room temperature and clarified by centrifugation. Total RNA was extracted from 200 μ l of pooled samples obtained from 5 swabs (2 pools/flock) using Qiagen RNeasy Mini kit. For rotavirus detection RT-PCR assay aimed at NSP4 gene was used (Day at al., Avian Dis., 2007).

Rotaviruses were detected in 16,1% (26/161) of analyzed Polish flocks: 10 positive flocks/80 (12,5%) in 2008, 10/56 (17,8%) in 2009 and 6/25 (24%) in 2010.

The NSP4-gene positive products from 23 rotavirus-positive samples were sequenced and compared with relevant sequences available in GenBank. Phylogenetic analysis revealed that all Polish rotaviruses clustered in one group together with Ty-3 strain isolated from turkeys in United Kingdom in 1979. Two additional clusters among turkey rotaviruses were formed by viruses previously detected in USA, described as "upper Mid-West" and "North-Carolina" subgroups. NSP4 gene sequences of rotavirus isolated from chickens and pigeons (Ch-1 and PO-13, respectively) were distinctly different from analyzed sequences of turkey isolates and formed separate group. The Polish rotaviruses had nucleotide sequence homology between 95.1 and 100% among themselves, 96.7 to 98.1% with reference Ty-3 strain and 91,8-97,0% with "North-American" rotaviruses. At the amino acid level, sequence homology rotaviruses from Poland were of 95.0 to 100% among themselves, 96,9 and 99,4% with Ty-3 and 93,7-96,2 with "North-American" rotaviruses. Results of phylogenetic study may illustrate the active evolution of turkey rotaviruses strains. In spite that some isolates were from healthy flocks it could not be precluded that rotaviruses caused observed health problems and poor performance of meat-type turkey flocks in Poland.

D 32: PREDICTING THE PROBABILITY OF INFECTION WITH AVIAN INFLUENZA VIRUS (VIRSU ISOLATION) AND CORRESPONDING TITERS BASED ON QUANTITATIVE RT-PCR RESULTS

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 CVI^1

Key words: Influenza, diagnostics, H5N1, PCR, virus isolation

Lately, real time quantitative PCR is used more often than the virus isolation techniques to study pathogenesis and transmission of avian influenza virus. With PCR it is possible to detect lower amounts of virus than with virus isolation methods. However, infected animals (naïve or vaccinated) will develop (or have increased level of) antibodies following infection. Thus, it is not clear how much of virus detected by PCR represent viable infectious virus and are not genetic material coming from neutralized virus or defective virus particles. In this study we developed two multivariable regression models, which can be used as tools to predict the probability of virus isolation as a function of the sample equivalent PCR titer. Results from two vaccination challenge experiments using HPAI H5N1 virus were used to develop (first experiment) the models and to validate (second experiment) them. The results show that in addition to the PCR titer, the type of swab sample (trachea or cloaca) and the day postinoculation (dpi) - of the experimental bird - the sample was taken, contributed significantly to the prediction of positive virus isolation and extrapolation of its expected titer. Using the model predicted values, ROC curves were fitted and a threshold (cut off) value was selected. Using this threshold, the sensitivity of the model to predict virus isolation was 98% and the specificity was 80%. The minimum PCR titer required to have a positive virus isolation increases with the dpi. Eq. a trachea swab, taken 1 dpi would need to have a minimum PCR equivalent titer of 0.9 EID50. If taken 2 dpi, the PCR titer has to be 1.1 EID50, etc. The model results show that the difference between PCR and virus isolation increases the closer the animal is to recovery (higher antibody titers). These models can be used to adjust the PCR results of experimental studies.

D 33: RAPID PRE-CLINICAL DETECTION OF CLASSICAL SWINE FEVER BY REVERSE TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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LVRI^1

Key words: Classical swine fever virus (CSFV); Reverse transcription of loopmediated isothermal amplification (RT-LAMP); pre-clinical detection

Classical swine fever (CSF) is a highly contagious disease affecting swine and resulting in severe economic losses, characterized by fever, neurological disorders, hemorrhages, and high mortality rates. The etiological agent, classical swine fever virus (CSFV), is a member of the genus Pestivirus, which belongs to the family Flaviviridae. CSFV is a small, enveloped virus with a 12.5-kb positive, single-stranded RNA genome containing a single large open reading frame encoding a polyprotein precursor, which is cleaved co- and post-translationally by cellular and viral proteases into structural and nonstructural proteins. Both ends of the genome are flanked by 5' and 3' untranslated regions (UTR), which are highly conserved among all of the virus isolates. The 5' UTR functions as an internal ribosomal entry site for translation initiation of the pre- polyprotein and genome replication.

Up to now, CSF has not been completely eliminated in the world and its outbreaks reported from time to time in some countries or regions. A rapid diagnosis based solely upon clinical signs is difficult and can lead to late detection. Rapid and pre-clinical laboratory diagnosis of CSFV is therefore a matter of urgency in order to prevent and control the epidemics. Current methods for diagnosis of CSFV rely on virus isolation, fluorescent antibody technique, enzyme-linked immunosorent assay, RT-PCR and real-time RT-PCR. RT-PCR and real-time RT-PCR procedures are generally considered to be the most sensitive in vitro method for detecting CSFV infection. However, this technique requires centralized laboratory facilities and clinical specimen submissions, which delay disease diagnosis, thus affecting the efficiency of emergency disease management.

Loop-mediated isothermal amplification (LAMP), a novel amplification method, has the ability to amplify specific DNA sequences under an invariable temperature between 63°C and 65°C and enable visual judgment within 60 min, besides its highly sensitivity and specificity. The method had been successfully applied to detect human influenza A virus, severe acute respiratory syndrome coronavirus and Japanese encephalitis virus. In our study, the usefulness of reverse transcription LAMP (RT-LAMP) for rapid pre-clinical detection of CSFV infection was evaluated. The RT-LAMP reaction could be finished in 60 min under isothermal condition at 65°C by employing a set of four primers targeting the 5' UTR of CSFV. The RT-LAMP assay of CSFV showed higher sensitivities than that of RT-PCR, with a detection limit of 5 copies per reaction. No cross-reactivity was observed from the samples of other related viruses including porcine circovirus type 2, porcine parvovirus, porcine pseudorabies virus, Japanese encephalitis virus, and porcine reproductive and respiratory syndrome virus. The detection rates of CSFV RT-LAMP, RT-PCR and virus isolation for samples including blood, tonsil, nasal and rectal swabs from uninoculated pigs without any clear clinical symptom were 89%, 78% and 71%, respectively. Furthermore, all of the assays showed higher sensitivity for blood and tonsil swabs samples than nasal and rectal swabs. These results indicate that the CSFV RT-LAMP assay is a valuable tool for its rapid, cost-effective detection and has potential usefulness for rapid pre-clinical detection and surveillance of CSF in developing countries.

D 34: RAPID SUBTYPING OF H9N2 INFLUENZA VIRUS BY A TRIPLE REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

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$LVRI^1$

Key words: H9N2 influenza virus; Subtyping; Triple reverse transcription polymerase

Avian influenza A viruses are a public health threat world-wide because they are usually associated with severe illness and consequently a high risk of death. The viruses are enveloped, single-stranded negative-sense RNAs and classified further into subtypes 16 hemagglutinin (HA) and 9 neuraminidase (NA), respectively. To date, most HA/NA combinations have been identified in the domestic and wild bird reservoir. Among 16 HA subtypes, the H9N2 subtype of avian influenza viruses (AIVs) was detected first in the United States in 1966. In North America, there are no reports of H9N2 influenza virus associated disease in chickens, although these viruses can be found in wild ducks and have caused a number of outbreaks in turkeys. Reports from Europe, Asia and South Africa since the late 1990s indicated widespread distribution of H9N2 influenza virus. In terrestrial poultry of China, H9N2 influenza viruses were prevalent mainly in chickens and ducks.

Several molecular techniques, such as reverse transcription polymerase chain reaction (RT-PCR) and real time RT-PCR (RT-PCR), have been developed as rapid tests for AIVs. However, the single RT-PCR only recognizes one specific viral gene in one reaction and RT-PCR requires expensive or specialized equipment and reagents and are not applied easily in many small laboratories. A triple RT-PCR method was established for typing of avian influenza A viruses, and for subtyping simultaneously of H9N2 influenza virus.

The aim of this study was to develop a rapid, cost-saving triple reverse transcription polymerase chain reaction (triple RT-PCR) for subtyping H9N2 AIVs. The three primer pairs for amplification of target sequences of nucleoprotein (NP), HA and NA genes, respectively, were designed for subtyping the viruses in the triple RT-PCR. The sensitivity of triple RT-PCR was found to be 102 copies per reaction for each of NP, H9 and N2 gene. The specificity tests indicated that all of NP, HA and NA genes were positive for H9N2, only NP gene was positive for H5N1 and H1N1 AIVs, and the results were negative for the other avian viruses including Newcastle disease virus, infectious bronchitis virus, infectious bursal disease virus, duck hepatitis virus and avian encephalomyelitis virus. A total of 112 clinical samples were evaluated by the assay and the results showed that the sensitivity and specificity of triple RT-PCR were in accordance with the virus isolation. In conclusion, this method is rapid and cost-effective making it feasible and attractive for large-scale epidemiological investigation of H9N2 influenza virus.

D 35: SENSITIVE DETECTION OF HIGHLY PATHOGENIC PRRSV BY MULTIPLEX REAL-TIME RT-PCR

WERNIKE, KERSTIN¹; HOFFMANN, BERND¹; DAUBER, MALTE¹; LANGE, ELKE¹; BEER, MARTIN¹

 FLI^1

Key words: highly-pathogenic-PRRSV; Multiplex Real-time PCR

Porcine reproductive and respiratory syndrome (PRRS), one of the most economically important diseases of swine worldwide, is characterized by reproductive failure in pregnant sows and respiratory disease in piglets. The causative agent is the antigenically, genetically and pathologically heterogeneous PRRS virus (PRRSV), which is an enveloped positive-stranded RNA virus that belongs to the order Nidovirales, family Arteriviridae. PRRSV isolates can be classified into two distinct genotypes, the European (EU) and the North American (US). In 2006 a highly pathogenic strain of US-PRRSV (HP), causing a disease characterized by high fever, high morbidity and mortality, emerged in swine farms in most areas of China. HP-PRRSV, characterized by a unique discontinuous deletion of 30 amino acids in the non-structural protein 2 (Nsp 2), affected a large number of pigs thereby causing enormous economic losses.

A multiplex real-time reverse transcription polymerase chain reaction (RT-qPCR) assay for the simultaneous detection of PRRSV-EU and -US genotype as well as HP strain specific sequences was developed. Previously described EU- and US-specific RT-qPCR systems were adapted to available PRRSV strains. For the selection of HP-specific primers and probe published sequence information was used. To verify efficient RNA extraction and uninhibited amplification an internal control (IC) based on heterologous RNA was included.

Using dilutions series of RNA standards generated by in vitro transcription of synthetic oligonucleotides, it could be verified that in the multiplex approach the US- and HF-assays amplified in a linear fashion from 2.0E+06 copies down to 20 copies per µl with an efficiency of more than 98%. The EU-assay amplified the RNA down to 200 copies per µl. The sensitivity of each PRRSV assays in presence of the other genotype was tested in chequerboard titration using an EU and a HP strain. As HP strains are classified into the US genotype, amplification of both RT-qPCR systems, PRRSV-HP and PRRSV-US is observed. Even the simultaneous co-amplification of the opposed genotype with low Cq values hardly affected the sensitivity. The amplification efficacy of the internal control-RNA was not affected.

For validation of the multiplex RT-qPCR assay serum and different organ material from PRRSV posititive and negative swine were analysed. Furthermore sample material from animals, experimentally infected with the PRRSV-HP strain, were used for validation. These data as well as the results of sensitivity tests in comparison to a commercially available PRRSV RT-qPCR kit will be presented.

D 36: SERODIAGNOSIS OF ANTIBODIES SPECIFIC FOR SHEEPPOX AND GOATPOX USING RECOMBINANT CAPRIPOXVIRUS ANTIGEN ORF122S IN AN INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY

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 LVRI^1

Key words: Sheeppox, goatpox, ORF122S, ELISA

Introduction and Objectives

Sheeppox and goatpox, which can cause heavy economic loss in the endemic countries, are characterized by fever, generalized papules, internal pox lesions and death. The causative agents, sheeppox and goatpox virus, are the members of the genus Capripoxvirus in the family Poxviridae. Despite the considerable threat that these viruses pose to livestock production and global trade in sheep, goats and their products, convenient and effective serodiagnostic tools are not readily available. An indirect ELISA assay, based on virion envelope P32 expressed in Escherichia coli, has been used successfully for detecting antibodies specific for capripoxviruses from sheep and bovine. But the expression of stable recombinant P32 protein is very difficult. Another ELISA, used recombinant virion core proteins ORF95 and ORF103 for coating, has performed favourably to detect antibodies from the sheep and goats which infected virulent sheeppoxvirus (SPPV) or goatpoxvirus (GTPV), but it was unable to detect antibodies from vaccinated sheep or goats.

We have developed an sensitive and highly specific new indirect ELISA assay and able to detect specific antibodies in vaccinated or infected virulent SPPV or GTPV sheep and goats.

Materials and Methods

The major antigen region from ORF122, we named ORF122S identified by DNAStar Lasergen, was amplified by PCR and expressed in Escherichia coli as His-tagged fusion protein. The recombinant protein was purified by affinity chromatography and used for coating.

1) 180 sera as negative from sheep or goats without vaccinated or never infected capripoxvirus.

2) 28 sera previously characterize as positive by diagnosed clinically and PCR.

3) 36 sera as the positive from vaccinated sheep or goats.

Results

For 176 of the 180 sera previously characterized as negative, verified negative results by our ELISA assay.

The results of all 28 sera from sheep or goats infected SPPV or GTPV were positive.

All 36 sera from vaccinated sheep or goats yielded positive results by ELISA.

Discussion and Conclusions

These results have indicated that the indirect ELISA based on recombinant ORF122S has a satisfactory specificity and sensitivity. This assay is an useful and convenient tool to detect antibodies in sheep or goats infected virulent SPPV or GTPV and monitor the vaccination status.

D 37: STUDY ON A RAPID DETECTION TECHNIQUE OF DNA IN BRUCELLA SPP. FROM RAW MILK OF DAIRY COWS WITH BRUCELLOSIS

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$LVRI^1$

Key words: B.abortus, omp25 genes, nested-PCR, raw milk, detect

The nested-PCR for detecting OMP 25kd genes of Brucella abortus in raw milk from dairy cows was established for the first time in China. The technique is specific, by which only Brucella spp are dectected but neither Chlamydia psittaci nor Toxoplasma gondii or nor Campylobacter fetus is done; The sensitivity degree of this technique reaches 50-100cfu per ml dairy milk sample; It is proved that this method is both steady and reproducible through many tests. The milk specimens sampled from ten dairy cows positive for brucellosis with the serum agglutination test were examined by the nested-PCR and all of the milk specimens were positive also, the coincident between the nested-PCR and the serum agglutination test being 100%. Using this technique examination, the diagnostic result will be obtained within 24 hours. The method is able to be used for examining Brucella DNA in serum or other aborted pathologic materials sampled from raw milk of dairy cows with brucellosis also.

D 38: THE FIRST DETECTION OF AN EMERGING BOVINE PESTIVIRUS IN FOETAL BOVINE SERUM ORIGINATED FROM AUSTRALIA

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SVA¹; Swedish University of Agricultural Sciences²

Key words: pestivirus; foetal calf serum; contamination; biosafety

This report describes the first detection of an emerging bovine pestivirus, previously proposed as bovine viral diarrhea virus 3 (BVDV-3), in foetal bovine serum (FBS) originating from Australia. Bayesian analysis showed that the Australian strains shared a closer relation with these of South America origin but a distant relation to a Thai strain Th/04_KhonKaen. This study indicates that BVDV-3 may have been spread much widely than previously thought, and the findings have important implications in the safety of biological products used in the diagnostics as well as the production of therapeutics and vaccines.

D 39: THE SEQUENCE COMPARISON OF RHABDOVIRUSES ISOLATES FROM CARP (CYPRINUS CARPIO) REARED IN POLAND

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$NVRI^1$

Key words: carp, Rhabdovirus, sequencing

Thirteen virus strains from carp (Cyprinus carpio) reared in different localities in Poland were isolated in at least two of the following cell line BF-2, EPC, RTG-2 or FHM. The isolates were identified using ELISA and immunofluorescens methods and classified as a members of Rhabdoviridae group.

The aim of sequencing which were being performed is to find out the degree of this group homogenicity and genogrouping of the rhabdoviruses actually present in carp in Poland, that were not made up to this time.

The investigations were based on the amplicon consisting of 550 nucleotide G gene and RT-PCR were used to amplify this part of genome. We have analysed samples by sequencing and compare our isolates with data base GeneBank.

D 40: TOWARDS AN HA ANTIBODY DIFFERENTIATING ELISA FOR SEROLOGIC SURVEILLANCE OF AVIAN INFLUENZA VIRUS

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Key words: avian influenza, surveillance, ELISA

Surveillance of avian influenza virus is performed mainly by molecular diagnostic methods such as real-time RT-PCR, which can detect circulating viruses in a subtype-specific manner in specimens collected from presently infected animals. However, virus may remain undetected by this detection method due to PCR-inhibiting substances in the specimen, genetic shift mutations or immunologic clearance by the host. Serologic surveillance for detection of antibodies against avian influenza virus is more resistant to such problems and would give a more comprehensive knowledge about the virus prevalence in the analysed population, since currently as well as past circulating virus subtypes could be traced. Such serologic differential surveillance tools for a broad range of influenza virus subtypes, based on ELISA are not yet available. Conventional tests suffer from an extensive cross reactivity of antibodies to the subtype-defining haemagglutinin (HA) antigens. In this study, we tested the concept of preabsorbing hyperimmune sera against different avian influenza subtypes with a broad range of HA subtype-specific antigens, to be able to set up an ELISA for detection and differentiation of antibodies against avian influenza virus subtypes. We have expressed recombinant haemagglutinin proteins of the HA subtypes H1, H3, H4, H5, H7, H11 and H12 from virus strains currently circulating in Switzerland, and used them as ELISA antigens and for immunization of chicken. In 850 tests, we compared different absorption strategies, using a total of 23 sera specific to these subtypes (4 for H1, 2 for H3, 3 for H4, 8 for H5, 3 for H7, 2 for H11 and 1 for H12). By preabsorption of the test sera with two different antigen concentrations with subsequent calculation of their difference in reactivity and by sorting out specificity-conserving subtype-groups, we could increase the initially low HA subtype specificity of 51% in a simple indirect ELISA system to 85%. This ELISA technique has not been described previously for antibody differentiation in avian influenza surveillance, and our preliminary validation data indicate the feasibility to differentiate a broad range of antibodies against avian influenza virus subtypes by means of an easy to perform and fast indirect ELISA.

D 41: VALIDATION OF FOOT-AND-MOUTH DISEASE VIRUS NON-STRUCTURAL PROTEIN (NSP) ANTIBODY ASSAYS FOR DETECTION OF INFECTION IN BUFFALO

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Murdoch University, Australia¹; IAH²; IZSLER³; FAO⁴

Key words: FMDV NSP antibody tests, buffalo, validation

In ruminants, persistent infection (carrier state) can be established following recovery of FMDV infection irrespective of vaccination status. Tests that detect antibodies against non-structural proteins (NSP) are used for sero-surveillance to assess the prevalence of FMDV carriers in cattle. These tests are not validated for detection of carrier animals in buffalo. There were direct evidence of transmission of virus from carrier African buffalo to cattle that caused two outbreaks in Zimbabwe during 1989 and 1991. However, in contrast to the carrier state in African buffalo (Syncerus caffer); very little information is available on the Asian and European buffalo as a potential reservoir of FMDV. Therefore sero-surveillance has been carried out in Lao PDR and Myanmar to assess the prevalence of FMDV carriers in Asian buffalos and to validate NSP tests that could detect carrier animals in buffalo. Four field trips to collect information and samples have been completed in September 2008, March/April 2009, October/November 2009 and May-June 2010. Laboratory analysis is being conducted at the Institute for Animal Health, Pirbright. 956 serum, probang and saliva samples were collected and evaluated in NSP antibody tests, Real-time RT-PCR and mucosal antibody test. In addition, 620 sera were received from Brescia, Italy, collected from Italian buffalo (Bubalus bubalis) that had been neither vaccinated nor exposed to FMDV. These were used to evaluate the specificity of the NSP serology. NSP 3ABC serology using 3 commercial and one in-house test have been completed on all sera collected from field studies, as well as on the Italian sera from FMD naïve buffalo. Serotype A and O viruses were isolated in cell culture from probang samples collected from Lao PDR and Myanmar respectively. Characterisation of NSP antibody test performances by Bayesian frame work revealed 60 to 80% of sensitivity and 97.9 to 99.5 % specificity. The detail data will be presented as power point presentation.

D 42: VALIDATION OF THE EPIZONE PANVIRALCHIP BY USAGE OF UNKNOWN SAMPLES

<u>ABENDROTH, BJÖRN</u>¹; HOFFMANN, BERND¹; HOEPER, DIRK¹; DASTJERDI, AKBAR²; BEER, MARTIN¹

FLI¹; VLA²

Key words: microarray, PanViralChip, diagnostics

Background

DNA microarrays are highly parallel biosensors which allow a simple and fast, standardized, simultaneously detection and identification of many differed pathogenic viruses in one or more samples. In the EPIZONE project, the PanViralChip (PVC) with 1150 different virus specific catcher oligonucleotides was created and submitted to the partners of working package 4.2 (microarray diagnostics) for further validiation. The idea of this cooperative work is to develop a fast and unspecific molecular diagnostic screening tool, to identify viruses in undefined samples.

Methods

For initial establishing and optimization of DNA microarray experiments self-made low density arrays were produced. Slide systems using clear and black substrate, different surface modifications and variant immobilisation systems were tested. DNA and RNA from classical swine fever virus (CSFV) and modified vaccine Ankara (MVA) were isolated from cell-cultures with different extraction kits. After first and second strand synthesis by using a cDNA synthesis kit (Roche), the amount of viral DNA and cDNA was increased by whole genome amplification (RepliG, Qiagen). The amplified DNA was shared by ultrasonic fragmentation and labeled with Cy3 and Cy5 (Nimblegen Cy3/Cy5 labeling kit, Roche). The labeled DNA was purified by precipitation with isopropanol and the hybridization was performed over night at 50°C. To reduce the background, different wash steps were applied to decrease unspecific bindings. By the usage of the Axon Genepix 3400A the slides were scanned. For the evaluation of preserved data a self-made R-program with different R-Software-pages were used. Based on the information obtained in these experiments, the validation of the PVC was carried out with different unknown samples.

Results

Based on the self-made low-densitity arrays optimization of slide handling, hybridization and labeling procedures will be presented. Slides with black substrate delivered the lowest background and the covalent immobilisation of amino-modified 70mer oligonucleotides showed the best hybridization values. In a first blind test for confirmation the functionality of the PVC, two "unknown" samples x and y were tested. After hybridization procedure and data analysis correct spots for the "un-known" Newcastle virus (NDV) and bovine enterovirus (BEV) samples could be identified on the PVC. Further validation data from the application of the EPIZONE PanViralChip using sample material from several animal species will be presented und discussed.
Epidemiology and surveillance

ES 1: AFRICAN SWINE FEVER SURVEILLANCE IN WILD BOAR POPULATION OF SARDINIA (2005-2010)

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Key words: Wild Boar, African Swine Fever, Surveillance

African swine fever (ASF) is a severe disease of pigs that affects all members of the family Suidae. The causative agent is a double-stranded DNA virus classified within the Asfarviridae family, genus Asfivirus. The infection appeared in Sardinia in the 1978 and, despite the systematic application of appropriate disease control strategies, the disease is still present. In central part of this island, pigs are farmed according to traditional practices, such as grazing free-range herds on vast communal lands. Free-ranging pigs are considered the primary reservoirs of ASFV in Sardinia – whereas wild boars probably play a secondary role. The role of wild Boar in the epidemiology of the disease is unclear: some authors have suggested that they are less efficient in transmitting the infection. However, in areas where the disease is actively circulating, where wild boars occur at high densities and where there are contacts with free-ranging pigs, they can represent a serious challenge as disseminators of the virus across different territories. In any case the surveillance of infection in wild boar populations is considered as a good indicator of the viral circulation.

Recently, the regional veterinary authorities decided to increase the representativeness of the surveillance in wild boars: a monitoring plan was implemented to investigate the presence of infection (by virological and serological methods) using a sampling collection based on geographical and epidemiological criteria. The regional territory was divided in macro-areas considering the distribution of wild boars meta-populations; the sections where the ASF infection was recently reported or suspected were considered as high risk areas and a sampling of at least 90 animals was recommended, whereas the territories where the infection was not recently evidenced were considered as low risk areas and a sampling of at least 58 animals was request.

Local hunting teams were enrolled in the samples collection and a specific informative campaign was implemented; data regarding the sex, the estimate age and other biological parameters of wild boars were registered; the official veterinary service controlled the progress of sampling activities while the laboratory investigations were performed by the Istituto Zooprofilattico Sperimentale of Sardinia.

The described program was applied with reliability during five annual hunting campaign; the cooperation of hunters with the veterinary service made available an acceptable samples number evenly collected in the regional territory. The sampling consistence was steady during the five years and a specific report of results was annually produced by regional veterinary observatory.

The analysis of these surveillance activities revealed a very low sero-prevalence with decreasing values in the last two years. During the 2007/8 hunting season 22 wild boars were found positives by virological methods, whereas were registered at most two positives in the other campaigns. All the positives samples were detected in the historical endemic area in the Nuoro province.

The ASF surveillance plan applied in Sardinia confirm the secondary epidemiological role that wild boars play in the persistence of infection. The continuous contacts between free-range pigs and wild population seems able to guarantee the maintenance of viral circulation in wild boars living in historical endemic area, whereas there are no evidence that the ASFV infection is transmissible toward separate meta-population living in the other territories of the Region.

ES 2: ASFv EXCRETION PATTERNS IN CHRONIC CARRIERS – A QUANTITATIVE APPROACH

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CVI¹; Utrecht University²

Key words: ASF, excretion, chronic

Introduction

When domestic pigs are involved, ASF (African Swine Fever) can have a huge economic impact. There is no vaccine available for this disease and the main strategies for control are animal quarantine and slaughter. Outside the African continent, the route of virus transmission is mainly through direct or indirect contact, without an arthropod vector being involved, and is therefore highly dependent on virus excretion by infected pigs. Key issues of virus excretion are virus amounts, excretion routes and duration of shedding. Some cases have been described where virus was detected in infected animals more than a year after the initial infection (DeTray, 1957, Mebus & Dardiri, 1980, Hamdy, 1984, Wilkinson 1983, Wilkinson, 1984). There is, however, a lack of quantitative data regarding the amounts of virus and duration of shedding, particularly in the period when apparently recovered pigs might become unapparent virus shedders (after 30 days post infection). In our study, we investigate excretion dynamics of 2 viruses, after inoculation in 2 different doses and after natural infection of contact animals.

Materials and methods

Three groups of 10 pigs each were used. In each group, 5 animals were inoculated while the remaining 5 were to be infected by contact. Two groups were infected with 2 different doses of the Malta78 isolate (3.0 log10 TCID50 and 4.0 log10 TCID50) and the third group with the Netherlands86 isolate (3.5 log10 TCID50). For a period of 70 days, samples were collected for virus titration (VT) and quantitative PCR. In two groups (Malta78 low and Netherlands86), air samples were taken to test for airborne virus.

Results

A carrier state was experimentally induced in 18 animals for an extended period of time (70 days). In the Netherlands86 group, only 3 out of the 5 inoculations were successful. The other 2 animals and the 5 contacts got infected approximately 10 days later, apparently all through contact infections with the three initially infected animals. In the Malta78 group infected with the lowest dose (3.0 logTCID50), also only 3 out of the 5 inoculations were successful. The other 2 animals and the 5 contacts got infected approximately 4 days later, again apparently all through contact infections with the three initially infected animals. In the Malta78 group infected with the lowest dose (3.0 logTCID50), also only 3 out of the 5 inoculations were successful. The other 2 animals and the 5 contacts got infected approximately 4 days later, again apparently all through contact infections with the three initially infected animals. In the Malta78 group infected with the highest dose (4.0 logTCID50), all 5 inoculated animals were successfully infected and the remaining 5 got infected approximately 7 days later by contact. Quantitative virus excretion patterns were obtained by PCR and VT, showing an initial peak between dpi 4 and 28, followed by prolonged virus excretion at low levels until the end of the experiment.

Air samples collected in the Netherlands86 group were PCR positive until dpi 25 and in the Malta78 (high dose) group until dpi 70. Detailed excretion patterns will be shown and discussed during the conference.

ES 3: AVIAN INFLUENZA IN THE MOEYINGYI WETLAND OF THE UNION OF MYANMAR: FINDINGS OF AN EPIDEMIOLOGICAL STUDY

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IZS-Ve¹; LBVD²; FAO Myanmar³

Key words: HPAI, field epidemiology, Myanmar, surveillance

Myanmar was officially infected by the H5N1 Highly Pathogenic Avian Influenza Subtype in March 2006 (first epidemic). Two other epidemics occurred in 2007 and were controlled by means of stamping out measures without vaccination. There were no follow up cases recorded in 2009 yet on 5 February 2010 an outbreak was notified in a laying farm in Yangon District. The Country has a wide range of agro-ecosystems and climatic diversity. One of the most important Avian Influenza risk areas is the Moeyingyi wetland that is located in the Bago East District: a wild bird sanctuary hosting 125 species surrounded by rice fields. On 19th October 2007 an outbreak occurred in a quail farm located in the vicinity of the sanctuary. Post outbreak surveillance was carried out in the traditional free ranging duck rearing sector. Explorative samplings provided insight into the complexity of the investigated issue which suggested a scientific approach was adopted. An epidemiological study was designed and was preceded by rural and appraisals. A census was carried out resulting in circa 3,823,340 ducks about equally distributed between the market oriented sector (1,769 duck farms) and backyards. The studied area extends over a surface of circa 1800 km2 and is sub dividable into four strata featured by common agro-ecological practices. (1) Moeyingyi wild birds sanctuary; (2) Roadside; (3) South lake earth-bund and (4) the channel district. Farms, either resident or make short transhumances, raise ducks for about two laying cycles. Flocks are confined during night-time and are conducted to water bodies (channels, ponds, the lake, flooded paddies) in day-time. A grazing diet is integrated with shrimps, broken rice and shells. Reproduction is centralised in 12 traditional hatcheries connected with occasional breeding farms. The duck sector is the most economically relevant income source for the area and produces 60% of ducklings and table eggs of the Country, thus being a potential route for the spread of the infection to other locations.

Forty farms were initially selected by means of a stratified random balances sampling. The selected farms were investigated by collecting 30 blood samples, 30 cloacal and/or tracheal swabs and by recording the occurrence of 11 risk factors. A cross-sectional investigation was carried out on 40 farms and allowed to identify a negative cohort of 18 farms which entered a longitudinal investigation performed across the length of an egg-laying productive season (October – May). Five samplings surveys were carried out. At the third sampling the size of the negative cohort decreased and new flocks were random selected and introduced to the study when negative. A total of 4,260 duck serum samples, 5,290 cloacal swabs and 1,060 tracheal swabs were collected in 142 flock samplings completed over 80 farms. Samples were tested at the Yangon National Veterinary Laboratory. The laboratory results produced in Yangon were compare with those of the same tests carried out at the AI/ND RL at the IZSVe and agreement was ascertained. Despite antibodies against the H5 AIV subtype were found, the virus was never isolated nor PCR for H5 AIV subtype was positive. The H5 AIV infectious dynamic is described through the interpretation of serological data. Risk factors distribution is analysed and statistical analysis performed.

ES 4: BOVINE AND SWINE LEPTOSPIROSIS IN M'ÐRĂK AND KHANH VINH DISTRICTS, VIETNAM: PRELIMINARY RESULTS

<u>NATALE, ALDA</u>¹; GAGLIAZZO, LAURA¹; BUCCI, GIULIO¹; VO THANH, THINH¹; LE, LAP¹; CEGLIE, LETIZIA¹; MARANGON, STEFANO¹; CRISTALLI, ALESSANDRO¹

IZS-Ve¹

Key words: Leptospirosis, Vietnam, zoonosis

Leptospirosis is a well known disease in pigs and humans in southern Vietnam. The monsoon climate in this region favours the persistence and transmission of Leptospires in the environment. Previous studies have shown that the leptospiral seroprevalence among sows was high in Southern Vietnam and that the highest seroprevalence was recorded for serovar Bratislava. The present study stemmed out from preliminary participatory epidemiological investigations carried out in the study area. High leptospirosis prevalence with consequent public health risk was suspected and further investigated in this study. The first aim is to assess Leptospiral infection among free-ranging cattle and pigs traditionally reared in two Districts of Central Vietnam, inhabited by ethnic minorities; the second aim was to consider the epidemiological correlation between cattle and pigs population due to their cohabitation.

In order to estimate the infection seroprevalence, a sample size for pigs and cattle population was calculated for each geographic District on the basis of the estimated populations and subsequently split into some villages characterised by similar conditions. A total of 166 bovine and 146 swine sera were collected and stored at -20°C until examination.

Serological analyses were performed by means of a Microagglutination test (MAT), based on 13 live antigen serovars (Icterohaemorragiae, Bratislava, Hardjo, Pomona, Tarassovi, Bataviae, Copenagheni, Canicola, Pyrogenes, Hebdomadis, Autumnalis, Grippotyphosa, Javanica), considered epidemiologically and geographically relevant. Cut-off titre was fixed at 1:100.

Furthermore, the size of the sample to detect the presence of Leptospira DNA in bovine and swine kidneys was calculated. At present, 114 swine and 10 bovine kidneys were collected and stored at -20°C until examination. Molecular analyses, with the objective to detect pathogenic Leptospires only, and are in progress.

Statistical analyses were performed with Software STATA 9.

Considering as positive any subject showing titres \geq 1:100 for at least one serovar, the overall seroprevalence was 74,70% in cattle and 45,89% in pigs, with a significant greater proportion in cattle (p<0,05). The same significant difference is maintained if the the cut-off value is shifted from 1:100 to 1:400 (seroprevalence 45,78% in cattle and 17,81% in pigs, p<0,05). With respect to the District and with a cut-off 1:100, a relation between District and seroprevalence is observed. In detail, Khanh Vinh District shows a higher infection risk for cattle (89.06% in Khanh Vinh, 65,69% in M'Đrăk), viceversa in M'Đrăk District pigs are at higher risk (62.20% in M'Đrăk, 25,00% in Khanh Vinh). Shifting the cut-off titre to 1:400, only cattle maintain a significant higher risk in Khanh Vinh District (65,63% in Khanh Vinh, 33,33% in M'Đrăk).

With regard to specific serological response, many serovars resulted abundant in cattle: Hebdomadis (33,73%), Icterohaemorragiae (33,73%), Pyrogenes (24,10%), Javanica (21,08%), Hardjo (19,88%), Bratislava (15,66%), Tarassovi (15,66%), Copenagheni (13,86%), Grippotyphosa (12,65%). Only 3 serovars resulted relevant for pigs: Grippotyphosa (12,33%), Icterohaemorragiae (11,64%), Hardjo (10,27%). These results highlight that each species had been in contact with serovars typical of the other one (for instance, high seroprevalence of serovar Hardjo in pigs, typical of cattle, and viceversa for serovar Bratislava) thus suggesting a mutual epidemiological risk due to species promiscuity. At present, data processing and molecular studies are in progress.

ES 5: DESCRIPTION OF AFRICAN SWINE FEVER VIRUS (ASFV) P72 GENOTYPE IX, A MAJOR CAUSE OF ASF IN DOMESTIC PIGS IN EAST AFRICA, IN CONGO WESTERN CENTRAL AFRICA

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Key words: African swine fever; p72; p54; CVR; genotyping

African swine fever (ASF) is a serious disease of domestic pigs caused by a DNA arbovirus belonging to the family Asfaviridae. Its highly contagious nature and ability to spread over long distances make it one of the most feared pig diseases causing devastating effects on pig production as it has been manifested in the Caucasus since its introduction from South East Africa in 2007. Considerable expansion of ASF has been reported in West Africa in the last 20 years and with the exception of Côte d'Ivoire the disease remains endemic. Due to a lack of discernible ASFV serotypes the field strains are grouped genetically using sequencing of the C-terminus of the p72 protein identifying 22 genotypes. Genotype I is historically associated with West African outbreaks, whilst Southern and Eastern African viruses have higher heterogeneity with all 22 known genotypes as so far recorded.

The Republic of Congo is located in the central-western part of sub-Saharan Africa sharing borders with Angola exclave of Cabinda, the Democratic Republic of the Congo, the Central African Republic, Cameroon and Gabon. The last OIE-reported ASF outbreaks occurred in Congo were declared in 2003. Since then, the disease has been officially declared as endemic but without quantitative data on prevalence placing a significant proportion of the Congo pig population at risk.

On August 2009, an organized FAO mission was undertaken with counterparts in Congo to support the development of an action plan for control of ASF. From the survey conducted, 86 samples were collected in 6 of 10 administrative departments which represent between 80-90% of the national pig population and were submitted at the CISA-INIA, Madrid, Spain, for confirmatory diagnosis and characterization of the ASFV strain(s) responsible for the outbreak(s).

The presence of ASF was confirmed in five out of the six departments where survey was conducted. Five ASFV Congo isolates were genotyped identifying the presence of genetically distinct viruses circulating simultaneously in the country. In East Congo districts, viruses were related to those recovered from the outbreaks occurred in Kenya and Uganda in 2006 and 2007. This is the first description of East Africa genotype IX in West Africa and strongly suggests recently widespread transfer of viruses from East to West Africa, most likely through uncontrolled movement of livestock across Central Africa. In contrast, western Congo viruses were associated with genotype I viruses historically circulating in West Africa. Analyses of the CVR identified two discrete variants within genotype I. The Pointe Noire ASFV was identical to historic viruses isolated in neighbouring Angola in 1970's, indicating a prolonged persistence of this virus type in the region. Whereas, the ASFV isolated from Niari was related to variants circulating in 1980's in Burundi associated with sylvatic cycle transmission, involving wild suids. Although is unknown a significant wild pig movement between East and West Africa, these results are consistent with dissemination of ASF from persistently infected wild animals to domestic pigs, which have then moved from East to West Africa. It is therefore important to study the ASF in Central Africa with an emphasis on countries such as DRC.

ES 6: DETECTION OF BOVINE LEUKEMIA VIRUS (BLV) INFECTION IN BOVINE IMMUNODEFICIENCY VIRUS (BIV) SERO-POSITIVE CATTLE IN DAIRY HERDS

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Key words: BIV, BLV, Dairy cattle, Iran

The role of Bovine immunodeficiency virus (BIV) infection in predisposing to other animal diseases remains controversial. Encephalitis, lymphadenopathy, lower milk yields, and immunodeficiency have been associated with the virus, but have been difficult to replicate in experimentally infected calves. Since the original viral isolate in 1972, three further isolates have been confirmed worldwide. No isolates have been reported to date from Europe. In spite of high prevalence of BIV infection in brucella sero-positive cattle, we couldn't find any significant correlation between two infections in our previous study. In this study we try to find any correlation between BIV and Bovine leukemia virus (BLV) infections.

For this purpose 2000 cattle sera had been screened from 90 dairy herds in center of Iran for detection of anti-BIV antibodies by ELISA test. Also sero-positive samples were confirmed by detection of gag and env proviral DNA sequences. All BIV sero-positive and 55 sisterhood cattle sera, randomly were selected from sero-negative animals used as a control group, were tested for detection of anti-BLV antibodies. Co-infection with both BIV and BLV, single infection with one of them and non-infected animals among four groups, statistically were analyzed by Cochran-Armitage test.

We could find 35 BIV sero-positive samples among all sera. The rate of infection was 1.75% in all farms. Out of 90 samples 12.2% (n=11) showed co-infection with BIV and BLV. Only one sample was BLV positive among 55 BIV sero-negative sera.

BLV is an oncogenic virus which has worldwide spread in cattle and it has an important problem with high level of costs to the dairy industry. Serological surveys in cattle in Iran indicate broad spectrum prevalence rates of 1.2-91% within herds. BIV infection was reported in many countries around the world and it seems that this emerging virus spread rapidly with undetermined losses because of retroviral characteristics and behavior like HIV. Sero-prevalence rates of BIV have been reported between 1.4% and 64%, but mostly in the range of 4% to 6%.

In this study, there was a highly significant correlation between BIV and BLV infections in this area. As both retroviruses have immunosuppressive effect and long life infection, they might be having a role in predisposing other infection. The varied cellular tropism of the virus, appearing to infect lymphocytes and monocytes suggests multiple potential mechanisms for the induction of immunosuppression.

In the other hand, almost retroviruses have the same routs of transmissions and follow same epidemiological pattern so this association may be result in neglected same transmission way. More studies need to find more details but these results encourage us to think about eradication of BIV from dairy herds, now, before encountering to widespread and persistent infection in future.

ES 7: DETECTION OF WEST NILE AND USUTU VIRUSES IN FIELD COLLECTED MOSQUITO IN 2010 (EMILIA-ROMAGNA REGION - ITALY)

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Key words: mosquito, West Nile virus, Usutu virus

Background

In recent years human diseases due to mosquito-borne viruses were increasingly reported in Emilia-Romagna region (Italy) - chikungunya virus in 2007, West Nile virus (WNV) in 2008 and Usutu virus (USUV) in 2009 - suggesting the need for an investigation to determine the presence and geographic distribution of these arboviruses. A mosquito based survey was started in late summer 2007, and continued every summer until 2010.

Methods and Materials: Mosquitoes trapped by modified CDC traps baited by CO2 were pooled according to date, location and species, with a maximum number of 200 specimens per pool. The pools were grinded and centrifuged and an aliquot of supernatant was collected, RNA was extracted and retro-transcribed then analyzed by different genus-PCRs targeting flaviviruses, alphaviruses and orthobunyaviruses and specific-PCRs targeting WNV and USUV. The obtained amplicons were sequenced.

Results

A total of 438,558 mosquitoes, grouped in 3,111pools, were analyzed in 2010. The most tested species resulted Culex pipiens (90.9 % of total mosquitoes), followed by Aedes caspius (4.1 %), Ae. vexans (4.0 %) and Ae. albopictus (0.4 %).

The survey allowed the detections of WNV in 3 pools of Cx. pipiens, and USUV in 89 pools of Cx. pipiens and 2 of Ae. albopictus. Both WNV and USUV sequences show a high identity between themselves and with the sequences obtained in previous years from mosquitoes, birds and humans.

Furthermore sequence data showed the presence of mosquito-only flavivirus RNA in tested mosquitoes.

Conclusion

The 2010 data confirm the persistence of WNV and USUV in the region, after their detection in previous years; the similarity of sequence obtained indicate the probable overwintering of the two viruses. In 2010 circulation of both viruses was lower and later than in 2009, probably due the different climatic condition, but presence of USUV was very relevant (91 positive pools). This observation highlighted the necessity to survey the presence and the diffusion of USUV

(as well as those of WNV) considering the potential pathogenicity of this virus for man (at least in immunocompromised individuals).

ES 8: DEVELOPMENT OF A MULTI-PATHOGEN SERODIAGNOSTIC ARRAY

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Key words: microarray, serology, WildTech, ELISA

A multi-pathogen protein array is being developed to enable serological surveillance of emerging and re-emerging infections in wildlife, as part of WildTech, an EU-funded project. In the last few years 75% of diseases which have emerged have arisen in wildlife, and a tool that can screen samples for exposure to multiple pathogens would be of great benefit. In addition, the system has the capacity for additional antigens to be added where new targets are identified.

A first-generation array containing 69 different antigens preparations from 50 bacterial, viral, and parasite pathogens has been produced (Alere Tech). Antigens used include crude boiled lysates, LPS, flagellar and surface antigens and pure, recombinant antigens. Antigens were spotted in triplicate on the array at 2 different dilutions. A chimaeric protein A/G –HRP (ThermoScientific) conjugate is used in detection in order to overcome the problem of needing multiple species-specific secondary antibodies.

To date, arrays have been tested with a panel of known positive and negative sera raised against a range of pathogens. Positive sera include both hyperimmunised and infected animals. The same antigens were also tested by ELISAs for comparison. Antigens and antisera were obtained from Veterinary Laboratories Agency, WildTech partners, commercial companies, and by donation.

Initial studies with this first generation array using C. jejuni-specific antisera have given promising results, with a clear positive signal and little cross-reaction, with results corresponding well to ELISA results. However, some antigens (e.g. BVD ELISA antigen, Mycobacterium avium ss paratuberculosis CFT antigen, Yersinia enterocolitica crude cell lysate antigen) produce a very low level or no array signal when tested with hyperimmune sera and therefore a direct comparison could not be made with ELISA.

Initial studies appear to suggest arrays are less sensitive than ELISAs, however, this may be a reflection of the quantity of antigen used in each assay and also covalent immobilisation of antigens on the microarray might affect sensitivity of the tests when comparing with ELISA. Further array generations have the potential to overcome this problem. A comparison of ELISA and array data using sera raised against a number of common pathogens will be presented at the meeting.

ES 9: DO THEY MEET OFTEN? – GENETIC SIMILARITY BETWEEN EUROPEAN POPULATIONS OF A POTENT DISEASE VECTOR CULEX PIPIENS

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Key words: mosquito, vector, genetic similarity, disease dispersal, Culex pipiens

Mosquitoes are vectors for a number of important insect-borne diseases that can infect both humans and domesticated animals. In Southern Europe the mosquito Culex pipiens is a competent vector for several types of pathogens of great medical importance, such as the West Nile Fever Virus (WNFV), Rift Valley Fever Virus (RVFV), and Eastern / Western Equine Encephalitis Virus (EEEV / WEEV). Ongoing climate change combined with increasing globalization makes it possible for pathogens previously absent in Northern Europe to establish themselves in new areas. One way to estimate the rate of contact between mosquito populations that carry certain pathogen dispersal, is to look at the gene exchange between mosquito populations. To measure the genetic diversity between European Culex pipiens populations we used 8 microsatellite markers in 10 populations of Culex pipiens originating from northern, central and southern Europe. Our data showed an extensive amount of gene exchange between European Culex populations with only a few populations being isolated. We therefore conclude that pathogens presently occurring in southern areas are likely to disperse northward with favorable climatic parameters.

ES 10: EMERGENCE OF A NOVEL H1N2 SWINE INFLUENZA REASSORTANT STRAIN IN PIGS DERIVED FROM THE PANDEMIC H1N1/2009 VIRUS

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Key words: swine influenza, reassortant H1N2 strain, pandemic H1N1/2009 virus, pig, Italy

Swine influenza (SI) monitoring programs have been carried out in Italy since the 1990s, and from 2009 testing for the pandemic H1N1/2009 virus (H1N1pdm) was also performed on all the swine samples positive for type A influenza. In May 2010, mild respiratory symptoms were observed in around 10% of the pigs raised on a fattening farm in Italy. Lung homogenate taken from one pig showing respiratory distress was tested and resulted positive for Influenza type A and H1N1pdm by two real time RT-PCR assays. The novel SIV isolate (A/sw/It/116114/10) was obtained from a 10% lung homogenate inoculated onto Caco-2 cells and into SPF chicken embryonated eggs. A multiplex RT-PCR was used to subtype culture supernatant, allantoic fluid and lung tissues that unexpectedly resulted to be H1pdmN2. Phylogenetic analysis, based on the complete genome sequencing, showed that all genes of A/sw/It/116114/10, except NA, belonged to the H1N1pdm clusters. Phylogenetic tree of the HA gene showed that A/sw/It/116114/10 grouped together with H1N1pdm sequences in cluster 7, like most of H1N1pdm human isolates in Italy (2). The NA was closely related to two H1N2 double reassortant swine influenza viruses (SIVs), previously isolated in Sweden (A/sw/Sweden/1021/09) (1) and Italy (A/Sw/It/58769/10). NA sequences for these three strains were clustering with H3N2 SIVs. The identification as a novel H1N2 reassortant strain was confirmed by full length sequencing of the HA and NA genes performed directly on lung homogenate. Full genome sequencing of their internal genes confirmed a very high similarity (from 99-100%) through these genes to H1N1pdm.

The identification of this H1N2 reassortant virus demonstrates the important role played by pigs as mixing vessels for animal and human influenza viruses (IVs), providing a place for reassortment and host adaptation to take place. The introduction of H1N1pdm into the swine population could provided opportunities for reassortment with the risk that the H1N1pdm virus could alter transmissibility and increase virulence. The success of interspecies transmission of IVs depends on the viral gene constellation. Successful transmission between species can follow genetic reassortment with a progeny virus containing a specific gene constellation with the ability to replicate in the new host. The new reassortant virus could be supposed to have the capability to replicate efficiently in humans considering its specific gene constellation. This is characterized by 7 genes belonging to H1N1pdm, well-adapted to humans, and the NA gene closely related to H1N2 SIVs.

The isolation of H1N1pdm from pigs in different countries also raises further concerns on the possible establishment of H1N1pdm in the swine population. This could lead to a scenario with a co-circulation of IVs within swine that could act as mixing vessels for the reassortment between IVs of both mammal and avian origin with unknown implications for public health. All these facts could have a great impact in Northern Italy where over 70% of swine and 65% of poultry are raised and several epidemics of H5 and H7 avian IVs occurred in the last ten years.

Recently, SI surveillance in Hong Kong has given rise to the isolation of a reassortant virus with 7 genes derived from SIVs circulating in China and the H1N1pdm NA gene (3). These independent isolation events underline the importance of surveillance of IVs isolated in the swine population worldwide.

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ES 11: ENGINEERING FOOT-AND-MOUTH DISEASE VIRUSES WITH INCREASED GROWTH PROPERTIES AND ANTIGENIC MATCH FOR THE DEVELOPMENT OF IMPROVED VACCINE

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$LVRI^1$

Key words: Foot-and-mouth disease; Vaccine; Protection; reverse genetics

A licensed vaccine is currently not available against serotype A foot-and-mouth disease (FMD) in China since A/WH/CHA/09 was isolated in 2009, partly because it does not replicate well in BHK cells. Towards this end, a novel plasmid-based reverse genetics system was employed to construct a chimeric strain by replacing the nearly complete P1 gene in the vaccine strain O/CHA/99 with that in the epidemic stain A/WH/CHA/09. The chimeric virus displayed similar growth kinetics with its parental strains and was chosen as the vaccine candidate strain after 12 passages in BHK cells. Subsequently, immunization with the inactivated vaccine was conducted in cattle and humoral immune responses in most of vaccinated cattle were induced at day 7. The result of virus challenge by A/WH/CHA/09 at day 28 indicates that the group vaccinated by a 10 μ g dose was fully protected without sub-clinic infection. Taken together, our data demonstrate the chimeric virus with O/CHA/99 not only breaks through the bottleneck of propagation in BHK cells compared with A/WH/CHA/09 and provides excellent antigenic matching against serotype A FMD, but also is a potential marker vaccine to distinguish infection and vaccination, which suggests reverse genetics technology is a useful tool to engineer a vaccine candidate for FMD prevention and control.

ES 12: EPIZONE: DURATION OF PASSIVE IMMUNITY FOR SWINE VESICULAR DISEASE VIRUS IN PIGLETS BORN FROM EXPERIMENTALLY INFECTED PREGNANT SOWS

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IZSLER¹; CVI²

Key words: SVDV passive immunity

The purpose of the study was to provide information on the duration of passive immunity in piglets born from SVDV infected sows: knowledge of the endurance of maternally derived antibodies is essential in case of epidemics of SVD and may help to implement proper control measures.

A total of 10 pregnant SPF sows were infected with SVDV (0,5 ml/10^8.70). Sows were inoculated in the bulbs of the heel with an isolate originally obtained from the 2006-2007 SVD epidemic in Lombardy. At 3 dpi, all ten sows were lame, anorexic and reluctant to move and oedema was observed at the coronary bands. At 5 dpi, vesicular lesions were observed on the feet. Seven sows had lesions on the snout. Five pregnant sows did not carry on gestation, the remaining 5 gave birth to 41 piglets, 10 of which died. Sows' faeces were positive at day 3 and negative at 35 dpi. Sows were seropositive for IgM isotype antibodies at 7 dpi until 49 dpi as a maximum and for IgG isotype antibodies from 14 dpi onward. In piglets, neither clinical signs nor evidence of infection were detected. Decay rates were estimated, after log transformation of the antibody titers fitting a linear mixed-effects regression with piglets as random effect. Median survival times were estimated by Cox proportional hazard regression.

Piglets were seropositive for IgG isotype antibodies 3 days after farrowing and remained positive until a maximum of 193 for the first litter, 149 days for the second, 59 for the third one, 85 for the fourth and 90 days for the fifth litter. The mean log10 antibodies titre at 3 days of age was 2.86 (CI 95%: 2.71-3.01). The half-lives of the maternally derived antibodies were estimated at 23.4 days (CI 95% 22.2-24.7) for the first litter, 21.1 (CI 95% 19.1-23.6) for the second one, 8.9 days (CI 95%7.8-10.6) for the third litter, 24.4 (CI 95% 20.2-30.9) for the fourth one and 12.4 (95% CI 11.3-13.6) for the fifth litter. The median survival times were respectively 179, 164, 51, 93 and 69 days. The estimated overall median survival time was 90 (CI 95%: 66-140).

This study has showed the presence of maternal antibodies in piglets lasting at a minimum of 2 months to a maximum of 6 months of age: titres after 90th day are rather low getting closer to the cut-off value of the ELISA that is normally used to detect antibodies against SVDV. We conclude that a 3 months duration of passive immunity should reasonably be taken into account in surveillance program based on serological screening.

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

ES 13: EPIZONE: Fishpathogens.eu a new database in the research on aquatic animal diseases

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VET-DTU¹

Key words: Database, Pathogens, Fish disease

Www.fishpathogens.eu is a database developed during the Epizone project and maintained by the European Union Reference Laboratory for Fish Diseases1,2. The database was launched in June 2009 focusing on Viral Haemorrhagic Septicaemia Virus (VHSV)1 and in 2010 extended to include Infectious Haemorrhagic Necrosis Virus (IHNV)2. Here we present the ongoing work generating new database extension on Spring Viraemia of Carp Virus (SVCV), Koi Herpes Virus (KHV), and Infectious Salmon Anaemia Virus (ISAV) and the integration of a tool for an online genotyping of isolates. The objective of fishpathogens.eu is to collate and validate information on serious viral fish pathogens and organise it in an easily accessible way to be used for the scientific community. The information include data on animal species, geographic coordinates, time of isolation, mortality records at isolation etc combined with all available and validated genotype information and raw sequence data of the pathogen in question. The database design is based on freeware and could easily be implemented to include pathogens relevant for other species than fish. If some are interested in the platform we are happy to cooperate and share the database structure with other EPIZONE partners.

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 $NVRI^1$

Key words: pigeons, PPMV-1, epidemiology

Newcastle disease (ND) in pigeons, caused by an antigenic variant of the avian paramyxovirus type 1, spread worldwide during the 1980s causing panzootic in racing, show and feral pigeons. We established full-length nucleotide sequences of F and L genes of three pigeon paramyxoviruses type 1 (PPMV-1) strains isolated from feral and domestic pigeons in Poland in 2005-2010. We found two multibasic amino acid motifs at the F2/F1 cleavage site: 112RRQKRF117 (two isolates) and 112KRQKRF117 (one isolate); the intracerebral pathogenicity index (ICPI) ranged from 0.82 to 1.29 and therefore, based on the World Organization for Animal Helath (OIE) criteria, the isolates were classified as virulent.

Phylogenetic studies of 374 bp region of F gene revealed that two isolates: PPMV-1/PL/H20/10 (isolated from domestic pigeons) and PPMV-1/PL/332/05 (recovered from feral pigeons) were related and located very closely to each other on the phylogenetic tree (nt sequence identity 98.7%). Both isolates were classified into sublineage 4b (or genotype VIb), associated with "pigeon variants" of Newcastle disease virus. On the other hand, the isolate PPMV-1/PL/H2/10 (detected in ornamental pigeons) fell into sublineage 4a, largely composed of "ancient" fowl NDV isolates with only a few PPMV-1, showing very low nucleotide sequence identity with the other two Polish isolates (90.1-90.4%). However, based on its reactivity with a monoclonal antibody that specifically recognizes PPMV-1, the isolate was still classified as "pigeon variant" of NDV. The results of phylogenetic studies of L gene were similar to F gene analysis: the PPMV-1/PL/332/05 and PPMV-1/PL/H20/10 isolates were located next to each other on the phylogenetic tree (98.5% identity) while PPMV-1/PL/H2/10 isolate was distantly related (91.3 – 91.5% identity). The results clearly indicate: i) the existence of different sublineages of PPMV-1 in Poland; ii) a circulation of closely related PPMV-1 over a 5-year period; iii) an interchange of PPMV-1 between feral and domestic population of pigeons.

Acknowledgement: This work was supported by the EU Network of Excellence, EPIZONE (Contract No FOOD-CT-2006-016236), WP 6.2 "Molecular Epidemiology and Surveillance of AI and APMV".

ES 15: EPIZONE: MOLECULAR EPIDEMIOLOGY OF FOOTH-AND-MOUTH DISEASE VIRUS IN ASIA

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IAH¹; SAP²; LVRI³; National Veterinary Laboratory⁴; FAO⁵; IZSLER⁶; VET-DTU⁷

Key words: FMDV, sequencing, epidemiology, laboratory coordination

Despite control measures, Foot-and-mouth disease (FMD) outbreaks continue to occur in many Asian countries such as Turkey and China. In addition to local economic losses, these incursions threaten livestock industries in Europe that are FMD-free (without vaccination). The recent FMD outbreaks that have occurred during 2009 and 2010 in the Far East affecting the People's Republic of China including Hong Kong Special Administrative Region, Republic of Korea, Japan, Mongolia and the Russian Federation clearly demonstrate the transboundary nature of this disease. Nucleotide sequencing data can be used to define the genetic relationships between field strains of the virus and to monitor the spread of these FMDV lineages across country boundaries. Genetic characterisation based on VP1 nucleotide sequence data shows that the FMD viruses responsible for the field outbreaks in the Far East belong to the O/SEA/Mya-98 and A/ASIA/sea-97 lineages that are normally restricted to countries of mainland Southeast Asia.

In addition to routine VP1 sequencing methods, several FMD reference laboratories have recently developed a suite of protocols, mostly based on the use of complete capsid or genome sequences, to generate high resolution molecular epidemiological data. These data will form the basis of studies to define the spatio-temporal distribution of FMDV lineages in the region and will also provide insights into the evolutionary mechanisms by which new lineages arise in Asia.

The aim of this one-year EPIZONE internal-call project (IC4.7) was to facilitate the coordination of FMD virus sequencing studies between European and Chinese laboratories via the evaluation and dissemination of robust sequencing protocols, and sharing of sequence data for samples recently collected from the region. Eight complete genome sequencing protocols have been developed for O/ME-SA/PanAsia, O/ME-SA/PanAsia-2, O/SEA/Mya-98, O/SEA/Cam-94, O/CATHAY, A/ASIA/Iran-05, A/ASIA/sea-97, and Asia 1. These RT-PCR protocols were based on the amplification of 21 overlapping 500 to 700 nucleotide fragments, and shared a single panel of 11 primer pairs for the amplification of the region encoding the non-structural virus proteins.

Acknowlegement: This work was supported by EPIZONE (internal call IC4.7), FAO (Project PR 41764) and Defra (SE2938). Anyone who would like further details about this project, including technical information regarding the sequencing methods, please contact Don King at IAH (donald.king@bbsrc.ac.uk).

ES 16: EPIZONE: PHYLOGENETIC STUDIES OF H3 LOW PATHOGENIC AVIAN INFLUENZA VIRUSES ISOLATED FROM WILD MALLARDS IN POLAND

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NVRI¹

Key words: avian influenza, H3 subtype, wild birds

Avian influenza viruses (AIV) perpetuate in wild bird reservoir, mainly waterfowl and shorebirds. In our study we established partial or full-length sequences of all eight segments of four AIV isolates of H3 subtype detected in wild mallards in Poland in 2006-2010. The results were compared to the sequences of AIV available in public domain.

The major outcomes of the study are as follows: i) two isolates had unusual HA and NA segment combinations: H3N3 and H3N9 while the remaining two viruses belonged to more common H3N8 subtype; ii) all gene segments of the tested isolates were closely related to other low pathogenic AIV detected in Europe, Asia and Africa; iii) some of the genes of Polish H3 AIV were closely related to each other; iv) NP genes of two isolates were closely related to H5 low pathogenic AIV (LP AIV) detected in poultry in Europe; v) PA gene of one isolate (detected in 2010), even though not directly related, was grouped within the same phylogenetic group with PA genes of H5N1 highly pathogenic AIV (HP AIV) found in poultry and wild birds in Asia in 2008/2009 (98% identity, bootstrap value >90%).

Therefore, the results indicate that there is a constant interchange of AIV between wild birds in Eurasia and Africa (accompanied by reassortment events) with an occasional spillover to poultry. On the other hand, AI viruses can be maintained for a long time in a local population of wild birds. More interestingly, the analysis of PA gene suggests that some LP AIV detected in wild birds and H5N1 HP AIV responsible for recent outbreaks in poultry and wild birds in Asia share a common ancestor, a fact indicative of naturally occurring reassortments between LPAIV and H5N1 HPAIV.

These findings warrant a regular monitoring of AIV in the natural reservoir, irrespective of HA subtype.

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ES 17: EPIZONE: TRANSMISSION DYNAMICS OF BVDV-1 AND THE NOVEL ATYPICAL BOVINE

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Key words: BVDV, atypical pestivirus, transmission, cattle

Bovine viral diarrhea (BVD) is an OIE notifiable disease that has significant economic impact on the cattle industry world-wide. BVD is also an animal welfare concern; depending on the virus strain and host factors, the clinical manifestation of infection may vary from asymptomatic or mild to severe or even fatal disease. Consequences of infection may include reproduction failure, poor reproductive performance, and susceptibility to secondary infections due to the immunosuppressive effects of BVD. Persistently infected (PI) calves serves as a virus reservoir. BVDV is a pestivirus in the Flaviviridae family; BVDV-1 and BVDV-2 are recognized as unique species. Novel group of pestivirus, informally named BVDV-3 was described recently in bovine sera of South American and Asian origin. The presence of those atypical BVDVs in other continent is unknown, however without proper control measures, the virus contaminating veterinary reagents and vaccines could have spread further. Our study is the first one describing clinical outcome and dynamics of the infection of cattle with novel BVDV.

The aim of this study was to characterize the competition between BVDV strains in vivo during an experimental study in young calves. To investigate virus replication dynamics, possible recombination events, clinical signs, and virus transmission in calves infected with BVDV. Additional objective was to validate detection system for atypical BVDVs. The experiment included groups of five 5-6 months old, BVD negative calves. The groups were:

- 1. BVDV-1 group: inoculated with BVDV type 1
- 2. BVDV-3 group: inoculated with the novel atypical strain Th/04_KhonKaen
- 3. BVDV 1&3 group: inoculated with a mixture of BVDV 1 and 3
- 4. Control group: inoculated with Eagles MEM

Each animal in group 1-3 received 5 ml of 105 TCID50 of inoculum i.m. and 5 ml intranasally.

Serum samples from all the calves were tested for presence of virus and antibodies. The virus population and replication dynamics were quantified by real-time RT-PCR, sequence analysis. The calves were euthanized post infection day (PID) 42, necropsy performed and tissues collected.

Preliminary results indicate that experimental inoculations in all three groups were successful based on antibody response, virus shedding, clinical picture and blood analysis. Pyrexia was observed in BVDV-1 and BVDV-3 groups on PID 7-9 while in BVDV 1&3 PID 7-10. Statistically significant decreases in white blood cell counts were observed PID 5 in BVDV-1 and BVDV-3 groups; PID 7 in BVDV 1&3 group and PID 14 in BVDV-1 and BVDV 1&3 groups. Significant decreases of lymphocyte counts were observed in all virus infected groups on PID 2-5-7. Viraemia based on antigen ELISA on serum samples was observed in all three inoculated groups between PID 5 and 9 with the maximum O.D. values on PID 7. Immune response based on ELISA test for antibodies (O.D. values above cut off) was detected PID14 in BVDV-1 group and PID 21 in BVDV-3 and BVDV 1&3 groups. While O.D. values for BVDV-1 and BVDV 1&3 groups were similar, the readings for BVDV-3 were almost 50% lower between PID 21 and 42. Ongoing analysis is directed towards strain specific real time RT-PCR to quantify viral RNA loads in the blood and nose swabs, virus neutralization test against homo- and heterologous virus strains and virus detection in cell culture. Specific cellular and humoral immune response would be also evaluated based on cytokine and T-cell responses. The result will be presented.

ES 18: EQUINE VIRAL ARTERITIS IN HUCUL HORSES IN POLAND

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Key words: hucul horse, equine arteritis virus, prevalence, phylogeny

Equine arteritis virus (EAV) is one of the major viral pathogens of horses. It is the causative agent of equine viral arteritis (EVA), a contagious disease of horses and other equid species. Very little is known about epidemiology of EAV infection in hucul horses. The aim of the study was to determine the situation of equine arteritis virus (EAV) infections in hucul horses. A total of 176 horses (154 mares and 22 stallions) from the biggest hucul horse stud in Poland were tested. Antibodies against EAV were detected in 97 (55.1%) horses. The EAV seroprevalence among mares was 53.2% while in stallions - 68.2%. The percentage of positive mares increased with their age, thus amongst the mares of less than 2 years of age the percentage was 32.5%, while in the group of 3-5 years old increased to 59.4% and in the mares in the age of 6-10 years and older than 10 years 89.5% and 95% were seropositive, respectively. Among 11 seropositive stallions five were supposed to be shedders of EAV with their semen. It is likely that those persistently infected stallions were the reservoirs of the virus in the stud. Genetic studies using of ORF5 gene showed high homology between the viruses detected in the semen of those stallions what suggested lateral transmission between the stallions sharing the same stable. Persistent infection in an immature stallion, which has not yet been used for breeding, was established as a result of infection via respiratory route. Phylogenetic analysis confirmed that all hucul viruses shared the same ancestor and as most of EAV strains dominating in Polish horse population belonged to the European origin EAV subgroup (EU-1).

ES 19: EVALUATION OF ENV-GENE HRM ASSAY FOR GENETIC SUBTYPING OF SMALL-RUMINANT LENTIVIRUSES

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Key words: PCR, genotyping, small ruminant lentiviruses

Maedi –Visna virus (MVV) and arthritis-encephalitis virus (CAEV) of goats constitute the small ruminant lentiviruses group. The diseases caused by these viruses are worldwide spread, and represent a serious problem due to their latent course, the inevitable fatal outcome, and the lack of treatment. Differentiation of these agents is commonly based on the analysis of the genome, typespecific PCR techniques, nucleotide sequencing, restriction analysis, heteroduplex analysis.

For differentiation of MVV and CAEV the method of high resolution melting of the PCR products (HRM), which allows to detect single nucleotide substitutions in the studied sequence, was chosen.

In the current study, MVV strains such as M-88, K-796, and Tverskoy CAEV – isolate, described in previous studies (Sidelnikov G. et al., 2009; Burdinskiy V. et al., 2009) were used. Part of env-gene was amplified using degenerate primers to the variable region (positions 7653 – 7866 on Visna 1514 M60610.1). The PCR products were purified using Qiagen PCR purification kit (Germany). EvaGreen (Biotium, USA) dye is a green fluorescent nucleic acid dye with features that make the dye useful for high-resolution DNA melt curve analysis was used according to manufacturer's instructions.

High resolution melting analysis of env-gene PCR products from SRLV strains showed the presence of one peak for each sample, which indicates the presence of a particular reaction product. Range of temperature peaks corresponded to the average value of $83,1 \pm 0,3$ °C for strain M-88 and K-796, and for the Tverskoy isolate - $84,1 \pm 0,3$ °C. Different values of the temperature peaks for the samples confirmed the structural differences in the composition of the PCR products, thereby demonstrating their phylogenetic relationships. According to analysis of the temperature melting peaks, these strains were divided into two groups. In order to confirm the results obtained partial nucleotide sequencing of env-gene was carried out. Phylogenetic analysis confirmed clustering of the SRLV strains into two genogroups: group A - M-88 and K-796 strains, and group B - CAEV Tverskoy isolate.

Thus, using of HRM assay, is possible to determine the genogroups SRLV in the shortest period of time, and this method can be used for primary screening of field samples.

ES 20: FIRST ISOLATION OF MYCOPLASMA BOVIS FROM CLAF LUNGS WITH PNEUMONIA IN GANSU PROVINCE OF CHINA

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 $LVRI^1$

Key words: Mycoplasma bovis; isolation; identification; China

Mycoplasma bovis is a major pathogen causing respiratory disease, mastitis, and arthritis in cattle. It is believed that this mycoplasma species is responsible for sizable economic losses to cattle industry in both Europe and USA every year. China stands first level in the world in cattle population with around 106 million of head today. M. bovis was isolated only in 2008 after an outbreak of "shipping fever" pneumonia was diagnosed in Hubei province. In the present study, four mycoplasma isolates were isolated from four lung tissue samples of affected calves in Gansu province by modified Thiaucourt's medium. The isolates were identified as M. bovis by the biochemistry test, specific PCR test and sequence analysis of the 16S rRNA gene which is 99.3%-99.6% identity with type strain of PG45. Moreover, Seroconversion of 39 serum samples from affected calves was determined by MYCOPLASMA BOVIS ELISA KIT (BIO-K-260, Bio-X Diagnostics, Belgium).These results showed that M. bovis infection was present in Gansu province located in Northwest China, which is far from Hubei province. This finding is of epidemiological importance and indicates M. bovis infection may be widely spread in China. Impact on cattle industry of this disease should be further investigated and adequate control strategies should be timely designed in this country.

ES 21: FREEDOM FROM DISEASE: INCLUSION OF TYPE II ERROR IN SAMPLE SIZE CALCULATIONS

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 FLI^1

Key words: surveillance, imperfect-diagnostics, 'freedom from disease'

Random sample are often taken from a population to demonstrate the absence of a certain disease. From an economical point of view, the sample size should be as small as possible. However, the fact that the available diagnostic systems are imperfect must also be taken into account. Based on these conditions, it may be necessary to accept a maximum of positive diagnostic results for a sample size without having to reject the hypothesis that the study population is 'free from disease'. The statistical test for 'freedom from disease' is constructed usually by a given design prevalence and a confidence level which leads to a minimal sample size with a fixed maximum number of positive test results in the sample that still allows the conclusion that the population is 'free' from the disease.

The aim of the study is to verify whether the minimal sample sizes are appropriate to ensure the aims of the 'freedom from disease' concept under the assumption that the population is not diseased. It will be shown that there is a high probability that the hypothesis 'the population is free from disease' must be rejected, especially for small truly healthy populations. In these cases it is useless to choose the smallest sample size, also from an economic point of view, because in reality many healthy populations are misclassified due to the so- called type II error. The effect of type II error will be shown on a specific example of diagnostics and under different population sizes.

It is proposed to include control of the probability of the type II error in sample size calculations.

ES 22: HEPATITIS E VIRUS INFECTIONS IN DOMESTIC PIG AND WILD BOAR IN GERMANY – PREVALENCE AND EXPERIMENTAL INFECTION STUDIES

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Key words: HEV, prevalence, experimental infection, Germany

Hepatitis E virus (HEV) is a cause of acute clinical hepatitis among humans throughout developing countries. In industrialized countries an increasing number of autochthonous HEV infections have been identified, however, their transmission route remained mostly unknown.

HEV is a member of the genus Hepevirus of the suggested family Hepeviridae. The positive sense single-stranded RNA genome of approximately 7.2 kb contains three partially overlapping open reading frames (ORF1, ORF2 and ORF3). ORF1 codes for viral non-structural proteins containing methyltransferase, helicase and replicase domains, ORF2 codes for the viral capsid protein and ORF3 for a small phosphoprotein. Within the genus Hepevirus in mammals there are four human pathogenic genotypes, which are distributed to specific geographic regions: Genotype 1 has been found in human hepatitis E cases in Asia and Africa, whereas genotype 2 was first isolated from human cases in Mexico. Genotype 3 has been first isolated from human cases in Asia. Genotype 1 and 2 strains were exclusively detected in humans, whereas genotypes 3 and 4 represent zoonotic viruses with domestic pig, wild boar and deer representing the reservoir hosts.

In order to determine the current distribution of HEV in domestic pigs and wild boars in Germany, blood and liver samples of animals from different federal states were tested for the presence of HEV genomes and anti-HEV antibodies. For this purpose several real-time RT-PCR protocols were used for the detection of viral RNA and a commercial ELISA for detection of HEV-specific antibodies. Sequencing and subsequent phylogenetic analyses were performed to genotype and subtype the HEV strains prevalent in Germany. These investigations indicated a high but varying degree of seroprevalence and the co-occurrence of different genotype 3 subtypes in animals from Germany.

To investigate their susceptibility and a HEV-mediated pathogenesis, wild boars and mini-pigs were challenged intravenously with a wild boar derived HEV strain (genotype 3) from Mecklenburg-Western Pomerania. Blood and faecal samples of the infected animals and of untreated sentinel animals were collected periodically to study the onset of viremia, faecal HEV-RNA excretion and the start of the sero-conversion. Our data show for the first time that wild boar derived genotype 3 HEV can be transmitted domestic pigs easily.

ES 23: IDENTIFICATION OF NEW GENOTYPES OF AVIAN PARAMYXOVIRUSES TYPE I IN WEST-AFRICA PROVIDES NEW OUTCOME FOR PHYLOGENY RECONSTRUCTION

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Key words: Newcastle disease, Avian paramyxovirus, Phylogeny, Genotypes

Newcastle disease (ND) is one of the most lethal diseases of poultry worldwide. It is caused by an avian paramyxovirus 1 which presents a high genomic diversity. In the framework of an international surveillance programme launched in 2007 based on the collection of several thousands of samples on domestic and wild birds in Africa, Newcastle disease viruses were detected in apparently healthy fowls and wild birds. According to the molecular analysis of the fusion protein, around one third of the positive samples held a virulent genotype of Newcastle disease virus. Phylogenetic analysis based on the F and HN genes of six isolates recovered from poultry in Mali showed three subgenotypes that form a new genotype proposed here as genotype XIII. To confirm this, full genome sequencing of one isolate was generated and an extensive phylogeny reconstruction based on the amino-acid sequences of the different virus proteins and the six concatenated proteins was carried out using the neighbour joining, the maximum likelihood and the Bayesian inference method. A new genotype taxonomy for NDV is then proposed, including the creation of two new genotypes XII and XIII that complete the eleven genotypes described so far.

ES 24: MOLECULAR ANALYSIS OF THE B602L GENE OF RUSSIAN ASF VIRUS ISOLATES

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Key words: African swine fever, Polymerase chain reaction, High Resolution Melting,

African Swine Fever (ASF) is one of the most complex diseases affecting domestic animals. It is caused by a DNA virus belonging to the family Asfarviridae. Only pigs (domesticated and wild) are affected.

Since 2007 African swine fever outbreaks have been registered in Southern and Northern-Caucasian Federal regions of Russian Federation. High variability of virus genome and endemic epidemiological stuation in this area during last three years allow to consider biological and genetic changes among ASF virus isolates.

For the purpose to detect any genetic diversity of Russian ASF virus isolates comparative analysis of variable genome region B602L was performed. B602L gene is located in the central variable region (CVR) of ASF virus genome and codes nonstructural protein, which participates in virion assembly. This region can be used as a genetic marker of the first changes within ASF virus populations. In this study, to detect even single nucleotide substitutions in B602L gene high resolution melting (HRM) assay was used.

Following ASF virus isolates from wild boars and domestic pigs were chosen for futher research: Abkhazia-07, Kabardino-Balkaria-08,09,10; North Ossetia-08,09; Rostov-08,09; Krasnodar – 09,10; Stavropol'-08,09. According to previous research all ASFV isolates belonged to II genotype (Gallardo, 2009) and were identical on the ground of variable regions analysis (Elsukova, 2010).

Amplification of B602L gene was performed according to modified protocol published by Nix et al., 2006.

PCR cycling and HRM analysis were done on the Rotor-gene 6000 (Corbett Research). The intercalating dye used was EvaGreen (Biotium). The analysis was done with the software supplied with machine. The cycling protocol was 1 cycle of 95°C for 5 min, 35 cycles of 95°C for 10 s, 55°C for 25 s, 68°C for 20 s, 1 cycle of 68°C for 5 min, and a melt from 75°C to 95°C for all assays. The temperature was increased at the rate of 0,2°C with 2 s hold on each step. All reactions were done in triplicate.

Results of PCR cycling with EvaGreen a specific fluorescent curves for all samples were observed. After the cycling, PCR products were used for the subsequent HRM analysis. All ASF virus isolates had one peak on 85,1°C, and Tm values differed by only ~0,2°C. These were easily demonstrated by HRM analysis when the data were normalized for the fluorescence. Based on this result we could conclude absolute homology within this part of genome between all ASFV Russian isolates.

Thus, genotyping by HRM is a very reliable method that does not require confirmation by sequencing or other methods. HRM technique used in this study, decrease diagnostic test costs in comparison to standard protocols (PCR and further nucleotide sequencing) for virus identification. This is a good alternative to nucleotide sequencing which is currently used in the majority of laboratories. The HRM method is considered to be robust in investigations of genetic variability.

ES 25: MOLECULAR EPIDEMIOLOGY OF AVIAN INFLUENZA VIRUSES IN WILD BIRDS IN NORTHERN VIETNAM DURING YEAR 2006-2009

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Key words: epidemiology, avian influenza, Vietnam

Since the emergence of highly pathogenic avian influenza (HPAI) viruses subtype H5N1 in Asian countries including Vietnam, numerous efforts have been made worldwide to control outbreaks and eradicate the virus. The number of outbreaks caused by H5N1 subtype has decreased, however, incidences of H5N1 infections among poultry have still been reported sporadically in Vietnam. On the other hand, wild birds are suggested as a potential source for highly pathogenic avian influenza viruses in poultry. Therefore, to determine constellation of avian influenza virus among wild birds in northern Vietnam, surveillance was conducted during year 2006-2009.

In the period between 2006 and 2009, throat and cloacal swab samples obtained from a total of 650 birds were collected and inoculated into embryonated eggs for virus isolation. One strain with subtype H9N2 was isolated from a Hwamei (Garrulax canorus) in 2006, and a strain with subtype H5N2 was isolated from a Eurasian Woodcock (Scolopax rusticola) in 2007. In 2009, strains of H9N2 were isolated from a pigeon and six quails. Phylogenic analysis revealed that the HA genes of the strains isolated in 2009 belonged to the Ck/Bei-like lineage. On the other hand, the Hwamei isolate in 2006 belonged to the G1-like lineage and was closely related to the H9N2 human isolates obtained in Hong Kong in 2009. All other gene segments of this isolate were also closely related to the human isolate in 2009.

These results indicate that the human H9N2 virus is possibly originated from those circulating in wild birds. Monitoring of wild birds for avian influenza virus is important for prediction of new avian influenza outbreaks in Vietnam.

ES 26: MOLECULAR EPIDEMIOLOGY of SHEEP POX and GOAT POX DISEASES in NORTHEAST ANATOLIAN REGION, TURKEY

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Key words: sheep pox, goat pox, PCR-RFLP, Sequencing

Sheeppox and goatpox are viral diseases of small ruminants which characterised with outbreaks in wide gerographical region including Turkey.

It is aimed to investigate molecular epidemiology of sheep pox and goat pox diseases in Northeast Anatolian region of Turkey. For this purpose, sheep pox virus (SPPV) and goat pox virus (GTPV) DNAs were detected by PCR in 168 clinical specimens taken from 53 animals in 11 disease outbreaks. SPPV and GTPV differentiations were detected by PCR-RFLP and DNA sequencing methods.

All of the isolated virus samples from the outbreaks were determined as SPPV with PCR-RFLP method. This finding was further confirmed by sequence analysis of terminal region of the virus genome.

In the light of the findings of the present study, it can be cocluded that sheep pox and goat pox disease cases occur seasonally, especially in young animals, in Northeast Anatolian region. More importantly, the viruses isolated within the study period had SPPV genome charactestics, as determined by PCR-RFLP and sequencing.

ES 27: MOLECULAR TRACING OF VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUSES FROM DENMARK PROVIDES EVIDENCE OF MORE VIRAL CLADES AND CASES OF INTRODUCTION THROUGH LONG DISTANCE TRANSPORTATION

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Key words: Viral haemorrhagic septicaemia, rhabdovirus, molecular tracing,

Viral haemorrhagic septicaemia (VHS) is a serious rhabdoviral disease that infects rainbow trout. The disease may cause up to 80% mortality and is listed by the OIE as a notifiable disease. Based on the full length glycoprotein gene (G-gene) sequences previous studies have identified 4 robust genotypes of the virus, designated I - IV with a geographic basis for their distribution. Genotype I have been further divided into five sub-types designated Ia – Ie of which the fresh water VHSV genotype Ia is responsible for the majority of losses in rainbow trout aquaculture in Europe.

Denmark has been considered to be endemically infected by VHSV until 2009 where the country obviously has freed itself from the disease through a VHS eradication program. To study evolutionary dynamics of VHSV genotype Ia isolates in Danish aquaculture, the G-gene from all isolates causing outbreaks in Danish rainbow trout farms since 2004 have been sequenced. Phylogenetic analyses including these sequences and several isolates from outbreaks in other European countries showed that genotype Ia isolates can be further divided into a number of different clades of which one comprise Danish strains and another comprises strains from central European outbreaks. Coupling of phylogenetic and geographical information data was used to trace origins of virus populations and the spreading of the disease. Both spreading due to contact infection through water and by movement of fish was observed. The outcome from this study will constitute an important background for optimizing the design and strategies for prevention and surveillance of VHS.

ES 28: MULTIDIMENSIONAL AND GEOGRAPHICAL INTEGRATION OF VETERINARY-RELATED DATA BASED ON WAREHOUSE/GEO-DATA MART CONSOLIDATION TECHNIQUES

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IZS-Ve¹

Key words: Data warehouse, geo-data mart, data consolidation, GIS

When deploying a new information system, few organizations have the luxury of starting from a clean slate. In most cases, they need to recover data from legacy systems, enrich it with additional information, and transform it because their legacy and new data schemas do not match. This was evident in north-eastern Italy where, from 1997 to 2005, several different avian influenza (AI) epidemic waves occurred. Before these epidemic events, there was neither a corporate vision for AI epidemiological data nor tools to facilitate access to and dissemination of information among stakeholders. In addition, available data about poultry flocks were of poor quality and data integration among the different healthcare management departments involved in the poultry production system was scarce.

The different formats and structures in which spatial and non-spatial data are organised and distributed within the veterinary community may lead to non-optimal implementation of AI control policies. To provide the spatial and non-spatial data infrastructure needed to correctly manage veterinary interventions, distributed data needs to be integrated and made available for extensive analysis. In particular the goals of a veterinary decision support system are to:

• Integrate information from sources that have possibly a high level of heterogeneity and support read-only access.

• Provide extensive transformation capabilities in order to resolve conflicts between sources and in order to restructure source data into a desired target data mode.

• Decouple access to the integrated target data from the process of integrating and transforming data from sources into the target in order to allow for scalability and performance.

Data consolidation helps to provide an organisation with the single version of the truth from a wide range of source. A data warehouse combined with a geo-data mart (where the geo-data mart represents the "retail" level of the data warehouse) is a good solution to support the multidimensional analysis of veterinary data and enriched data exploration based on an explicit spatial reference represented on maps.

Three types of dimensions can be defined: the non-geometric spatial dimensions, the geometric spatial dimensions and the mixed spatial dimensions.

In this paper we present the 4-tier architecture developed by the Istituto Zooprofilattico Sperimentale delle Venezie for consolidated data warehousing combined with geo-data mart. The first tier is where integrated, homogeneous detailed data are extracted. The second tier represents a second data warehouse where the results of the aggregation processes are stored. The third tier is comprised of the data marts that contain subsets of specialised business data (that could be seen as "attributes" in a GIS environment) organised in a format that makes integration with the related feature entities (polygons, lines, or points) easy and efficient. These data marts that integrate the geographic information (geo-data marts) are processed and organized according to a vertical view of the data (e.g. within a range of map resolutions) or a horizontal view (e.g. within a region or a department). The fourth tier includes the clients that could be both desktop and web applications. Such architecture is particularly useful when the fusion of heterogeneous source data represents important efforts that cannot be fully automated.

Consolidation has been optimized to manage AI epidemiological data, but it is clear that the design principles can be extended to other diseases or surveillance activities.

ES 29: NS GENE BASED PHYLOGENETIC ANALYSIS OF THE EQUINE INFLUENZA VIRUSES CIRCULATION IN SWEDEN AND THEIR IMPLICATION IN IFN-BETA INHIBITION

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Key words: Influenza virus, Phylogenetic analysis, NS1 protein, IFN inhibition

Equine influenza (EI) is a contagious and economically important disease of equines. To determine the nature of circulating strains it is of paramount importance to study its prevalence not only for diagnostic but also for vaccine development. In the present study, we examined the prevalence and evolution of EI lineages in Sweden. Furthermore, the role of NS1 protein in IFN- β inhibition and its interference with NF- κ B and AP-1 promoter activations in different cell cultures was evaluated. Phylogenetic analysis, performed on the NS1 gene of seventeen samples indicated that MIA/63 (Miami/63)was considered as prototype isolate because it was the first one among the EI viruses isolated from Europe. Total five isolates were found to be close to European lineages while three isolates were closer to American lineages. Two samples exhibited a close relation with prototype MIA/63. One isolate was found near to FC2 lineages while five isolates were remained as uncharacterized. Notably, one sample was found closer to European lineage in contrast to previously reported American lineages. It was noticed that the NS1 protein has effect on interferon inhibition and this character expressed differently in different host cells. Three signaling pathways including ISRE (under the control of IFN-β), NF-κB and AP-1 were analyzed in MiLu, CEF and human A549 cell lines. A variation in IFN- β inhibition was observed. It was found that NS1 inhibits IFN- β in a descending order as measured by ISRE promoter inhibition in MiLu, human A549 and CEF. NS1 inhibited dsRNA dependent NF- κ B promoter activation which is best seen in MiLu but induced IFN- β in human A549. Since NS1 is a major determinant of influenza virus pathogencity, it is recommended to consider the variability of NS1 gene circulating in the area while designing vaccine and therapeutical agent.

ES 30: PREVALENCE STUDIES IN ROE DEER OF VECTOR-BORNE DISEASE SUPPORTED BY GAME MANAGEMENT UNITS

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Key words: BTV EHDV wild life seroprevalence deer

The population of roe deer (Capreolus capreolus) in the Netherlands has increased from +3,000 in 1930 to >60,000 today. The presence is scattered all over the country. The population is managed by 308 game management units (WBEs) collectively covering the Netherlands. WBEs obtain hunting permits from 14 fauna management units (FBEs). In 2009, a pilot study was started to improve the representativeness of roe deer in disease prevalence studies. WBEs and FBEs were requested for their participation in this study on bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV). BTV and EHDV, family Reoviridae, genus Orbivirus, are arthropod-borne diseases; transmission to ruminants, including cattle, sheep, goats, and deer, occurs by bites of species of Culicoides. Since 1998, BTV serotypes 1, 2, 4, 9, and 16 have invaded European countries around the Mediterranean Basin. Further, a severe outbreak of EHDV as well as new serotypes of BTV have recently been reported in Israel and Morocco. In 2006, BTV8\net06 (IAH collection nr. BTV-8 NET2006/04) invaded North-Western Europe resulting in the largest BT-outbreak ever recorded, therewith demonstrating the impact of these vector-borne diseases.

Five-hundred sampling packages were assigned to WBEs, using a random sampling system based on the number of deer counted per WBE. Volunteers took samples from roe deer shot in the Culicoides activity-free period between late 2009 and early 2010. Samples with date, location, sex, age class, health, and type of sample were collected. Blood samples were tested by PCR for EHDV, and by ELISA for antibodies (Abs) against BTV. The return rate was 73% (366 out of 500). Most of the samples were taken from the thoracic cavity, and were hemolytic. Samples were from does and calves, since bucks were not shot in this period. All 366 samples were tested negative for both EHDV and BTV. Though the representativeness of roe deer in the collected samples could be improved, this pilot study has shown an acceptable participation of WBEs and FBEs in roe deer disease prevalence for BTV in these mainly solitary living deer species. The implications for the role of roe deer in the epidemiology of vector-borne diseases, like the 'ruminant' orbiviruses transmitted by species of Culicoides, will be discussed.

ES 31: QUANTIFICATION OF AIRBORNE TRANSMISSION OF A HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS STRAIN H5N1 BETWEEN GROUPS OF CHICKENS

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Key words: Avian Influenza; H5N1; HPAI; transmission; chicken; airborne

Highly Pathogenic Avian Influenza (HPAI) is a devastating viral disease of poultry and outbreaks should be controlled as soon as possible after detection. One of the routes of transmission between flocks which is often reported is airborne transmission. More quantitative knowledge of the rate of indirect transmission via this route might contribute to the optimisation of control measures. In the current study, we quantified the rate of indirect airborne transmission of a HPAI H5N1 virus strain between chickens over various distances, under experimental conditions. In addition we quantified viral load in air and dust samples. Two experiments were carried out with 32, and 128 birds respectively. Chickens were housed as groups in cages. The first group was inoculated with strain A/turkey/Turkey/1/2005 H5N1. Other groups were housed at 20 and 100 cm distance of the first group, and were thereby indirectly exposed. This set up was carried out 16 times. Tracheal and cloacal swabs were collected daily to monitor virus transmission. Air and dust samples were taken daily to quantify virus load. Swabs and samples were tested by quantitative RRT-PCR and virus isolation. In 12 out of 16 set ups, no virus was transmitted from the experimentally inoculated chickens to the indirectly exposed chickens. No statistical difference was found in the rate of transmission at various distances. None of the air samples tested positive. Three dust samples were confirmed positive in virus isolation. This suggests that airborne transmission is not a very likely route of spread. Whether or not this also applies to the field situation needs to be examined.

ES 32: RABIES OUTBREAK IN MALOPOLSKA REGION IN POLAND – PHYLOGENETIC ANALYSIS OF ISOLATES

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NVRI¹

Key words: rabies, PCR-RFLP, phylogenetic analysis

Rabies is a zoonotic disease which occurs worldwide in many countries including Poland. The disease is transmitted by rabid animals during biting because the virus is contained in saliva of rabid animals. In Poland terrestrial animals are the main reservoir of virus, especially red foxes. To reduce a number of rabies cases two companies of oral vaccination per year are performed. The action started in 1993, two vaccines: Lysvulpen and Fuchsoral containing SAD Bern and SAD B19 strains, respectively, are used for foxes oral immunization. Since 2002 when the whole territory of Poland was covered with vaccine the number of rabies cases have decreased gradually each year. In 2008, 29 rabies cases were diagnosed whereas in 2009, only 8 rabies cases were reported. Western part of Poland is regarded rabies free since more than 10 years.

In summer 2010, after seven years, the outbreak of rabies appeared in Malopolska region (southern Poland). Since August to the end of November, 74 rabies cases were detected mainly in red foxes. Single cases were diagnosed in domestic animals including dogs, cats, cattle and horse.

The main goal of the study was the phylogenetic analysis of Polish rabies isolates collected from rabid animals in Malopolska region to asses the probable origin of rabies virus and to exclude of rabies vaccine strains as a source of epidemic.

The study included 74 rabid animal brains. Brains were homogenized in the water for injection (10% suspension) to conduct RNA extraction. RNA extraction was performed with the commercial kit QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instruction. As a template for PCR-RFLP, sequencing and phylogenetic analysis the most conservative fragment of 600 bp nucleoprotein gene have been amplified using One-Step RT-PCR Kit (Qiagen). For the differentiation of vaccine and street strains using PCR-RFLP method, Dra I restriction endonuclease was used. Sequencing was carried out in two direction with ABI sequencer. The consensus sequences were constructed after alignment in Clustal W software. Phylogenetic analysis was conducted using BioEdit software (on-line available) whereas phylogenetic tree was constructed using DNA maximum likelihood method and demonstrated using TreeView software.

PCR-RFLP of 74 RT-PCR products revealed characteristic pattern for street rabies virus eliminating the vaccine strains implication in rabies outbreak. The analysis of sequencing results as well as the phylogenetic analysis based on 570 bp fragments of nucleoprotein gene of vaccine and Malopolska isolates confirmed PCR-RFLP results.

Additionally, the phylogenetic analysis of Polish isolates and European rabies isolates available in GenBank database revealed the highest likelihood with NEE (North Eastern Europe) variants, among them the most similar are Romanian rabies isolates.

ES 33: RABIES OUTBREAK IN THE VENETO REGION: IMPLEMENTATION OF AN INFORMATIONS SYSTEM TO GATHER DATA ON VACCINATION IN DOMESTIC ANIMALS

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IZS-Ve¹; Regione Veneto²

Key words: Rabies, vaccination, computerized data collection

Following rabies outbreaks in wild animals in Northern Italy, in November 2009 the vaccination against rabies has been made compulsory for dogs living in the provinces of Belluno, Treviso and in 60 municipalities belonging to the provinces of Venezia and Vicenza. Similarly, dogs which are brought into or pass through these areas, and livestock, which are taken to mountain pastures in summer, must be vaccinated 21 days prior to entering these areas. Dog vaccinations had to be completed by March 31st, 2010 and had to be carried out at a fixed price both by Veterinary Services of 8 Local Health Units (VS) or by Veterinary practitioners, whereas livestock had to be vaccinated only by VS.

In the last 15 years, the Veneto Region has paid particular attention to the activation of computerized data collection systems in animal health, which is the prerequisite for the implementation of epidemiological surveillance networks laid down in Council Directive 97/12/EC.

To reach this aim, a centralized Data Bank (RDB) has been operating since 1997 in which data on herds, together with data on individual animals and their movements, are recorded. In this database, the health controls carried out by VS to avoid, control and eradicate infective diseases such as Tuberculosis, Brucellosis, Leukosis, are registered and related to each herd and to each animal. Moreover, a Regional Canine Registry Data Bank (BAC) has been implemented since 2003, in which the data and the movements of dogs, together with the personal data of the owner and the keeper of the animal, are stored.

The management programs respectively of the BAC and of the RDB, were updated and specific functions for the management of the vaccination plan were integrated in order to support the VS activity and to collect the data of dogs and livestock vaccinations in a centralized database. The packages developed allowed to:

- organize appointments for the owners of dogs to be vaccinated at a certain time, date and surgery;
- record vaccination information (brand and name of the product, batch number, date and place of vaccination, expiry date of immunization and Veterinary's name) referred to dogs, individual bovines, sheep and goats or to the whole herd;
- print the document confirming vaccination and the receipt of fees paid by the owner;
- calculate the amounts due to each Veterinary practitioner.

At present, the number of vaccinated dogs registered by VS in the BAC is 212,010 - about 75% of canine resident population-. There are 264 vaccinated herds recorded in the RDB, with 6,422 vaccinations of individual bovines and 411 of sheep, compared to 12,000 animals which usually go to pasture in these areas. In the latter case the percentage of registered vaccinations is lower because the RDB software began operation after the herds' departure for mountain pastures, whereas the dogs vaccination tool has been available since December 2009.

The use of the new packages and the computerization of data have helped in reaching the following objectives:

- automate the administrative activities involved in the organization of the vaccination plan;
- establish centralized collections of vaccination data, both homogeneous and correct;
- ready 'on line' availability of information on the vaccination status of individual animal or herd;
- supply periodical reports on the dogs' vaccination plan trend;
- supply data for evaluation of the level of vaccinal protection of the population;
- offer structured information to conduct epidemiological analysis and health checks (e.g. assessment of antibody level in vaccinated animals).

ES 34: RELATIONSHIP BETWEEN CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS STRAINS CIRCULATING IN IRAN AND TURKEY: POSSIBILITIES FOR TRANSBORDER TRANSMISSION

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Key words: CCHFV; S-segment; Phylogenetic analysis; Iran; Turkey

Crimean-Congo Hemorrhagic fever is a zoonotic viral disease that is asymptomatic in infected livestock, but a serious threat to humans. The fatality rate may be due to phylogenetic variation of the virus, transmission route, and efficient surveillance system for disease. Geographical features of eastern border of Turkey with Iran may facilitate transmission route of virus between countries of the region. Therefore in this study we have focused on genetic relationship between Turkish and Iranian CCHF viruses based on their S-segment sequences. The research was perfomed on a total 104 blood samples from small ruminants reared in south-west of Iran. The result of phylogenetic analysis showed that Iranian CCHFV isolates were found to be closely related to previously reported human originated Turkish Group II CCHFV in European lineage.

ES 35: SEROPREVALENCE OF BVDV IN SWEDISH SEMIDOMESTICATED REINDEER (RANGIFER TARANDUS TARANDUS)

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Key words: BVDV, pestivirus, seroprevalence, reindeer

BVDV infections are common in domestic ruminants around the world. Despite that the virus has been successfully eradicated in Scandinavian countries, wild animals as potential source of infection should be of concern. Blood samples from 891 reindeers (483 calves and 408 adults) from twelve reindeer herding districts in Northern Sweden were collected during the winter slaughter periods of 2001 and 2002. The samples were tested for antibodies against BVDV using ELISA. Additionally some selected sera were tested in seroneutralization test against different pestivirus strains. The study revealed an overall scroprevalence of BVDV of 34 %, in the reindeer population The seroprevalence of BVDV was clustered at the district level varying between 1.5 and 97%. The presence of antibodies was correlated to the age of the animal. The percentage of BVDV seropositive animals in the group of calves was 20% while in adult reindeers 57%. Very high seroprevalence to BVDV with the values of 95% of calves and 97% for adults in one of the herding districts may have been connected to the presence of persistently infected (PI) animals, however the existence of PI in this species needs further studies. Seroneutralization studies of selected ELISA positive sera against different pestivirus showed higher titers against border disease virus then BVDV. Whether the pestivirus circulating among reindeers was transmitted from domestic animals or is unique to the species needs further virus characterization.

On the basis of this study it can be concluded that pestiviral infections seem to be endemic in Swedish reindeer population. Since reindeer management practices are shifting towards more intensive animal rearing, in particular feeding during winter time, it is possible that infections with pathogens such as pestiviruses could be of greater consequence in the future.
ES 36: SURVEILLANCE FOR SWINE INFLUENZA IN POLAND IN 2010

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Key words: swine influenza virus, PCR, serology

Background

Swine influenza (SI) is an infectious, respiratory disease of pigs, worldwide distributed, and economically important. Pigs have an important role in interspecies transmission of influenza viruses therefore the regular monitoring of the epidemiological situation concerning SI is important for public health.

In Poland molecular and serologic survey is undertaken yearly, from 2000. In this report we present current situation and compare it with data evidenced during last 5 years.

Materials

Samples of pig sera: in 2010 5250 blood samples from unvaccinated animals raised in large pig farms located in whole country were tested.

Virus strains: SIV strains circulated among pig population in Europe of three subtypes: H1N1 (A/Sw/Bel/1/98), H1N2 (A/Sw/Eng/96) and H3N2 (A/Sw/Fl/1/98) were used.

Samples for virus/viral RNA isolation and molecular subtyping: in 2010 310 biological samples (277 nasal swabs, 33 lungs) taken from animals demonstrating influenza-like symptoms, raised in 23 farms, were used.

Method: hemagglutination inhibition assay (HI), RRT-PCR, multiplex PCR, phylogenetic analysis were performed.

Results and Discussion

Antibodies specific to H1N1 were detected in 1583 out of 5250 sera (30.15%). A survey for H1N2 subtype demonstrated 11.92% of seropositivity (626 positive samples), while the seroconversion to H3N2 antigen was detected in 825 out of 5250 tested sera (15.71%). Just a few samples (17) were positive for pH1N1 virus.

RNA of swine influenza virus was detected in 5 out of 23 tested farms.

In clinical specimens genetic material of SIV was identified in 13 nasal swabs. It was additionally detected in 9 lung tissues.

Using conventional virology method in total 9 new isolates of SIV were obtained.

In molecular subtyping all isolates were determined as H1N1 subtype. According to HA-gene phylogenetic analysis all new isolates were grouped together with the avian-like H1N1 isolated in last decade. The highest genetic similarity was observed with the strains currently circulated in Spain, France and Hungary.

Both monitoring study showed that in Poland H1N1 subtype is dominated.

The comparison of the epidemiological situation concerning the distribution of antibodies against SIV in the year 2004 and 2010 shows that prevalence of antibodies against subtypes H1N1 and H3N2 significantly increase in large farms, from 8.2% to 30.15% and from 2.2% to 15.71%, respectively). Antibodies against H1N1 and H1N2 was detected in most provinces of Poland. H3N2 antibodies were predominated in East part of the country.

It could be state that epidemiological situation concerning SIV in Poland is entirely different from that observed in USA (66.3%-100%) and Asia (45%). Situation of our pig herds concerning SI is similar to that evidenced in Europe (25%), especially in neighbouring countries, however with the lower prevalence. This situation might result from import of vaccinated animals from abroad. The dominated subtype in Poland is still H1N1, whilst in rest of Europe both, H1N1 and H1N2 viruses were isolated from several cases of SI.

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ES 37: SURVEILLANCE OF FISH DISEASES BY SEROLOGICAL METHODS

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Key words: Antibody detection, fish, VHS, IHN, KHV, IPN, ISA

Surveillance of fish diseases has hitherto been based on the detection of the pathogens alone, while antibody detection methods in only very few cases have been implemented for routine screening purpose. One reason is the significant influence of water temperature on the development of antibodies in fish, another is the relative simple IgM in teleosts which is more complicated to handle than IgG in homeotherms, with binding of lower affinity and avidity.

The advantages using serological methods are, however, significant, as the time scale in which an infection can be detected is usually much larger by serological methods than by antigen detection. Especially at high water temperatures antigens might remain undetectable short time after infection whereas antibodies will last for up to 1 year. Another advantage is the possibility for non-lethal testing as a large number of fish are sacrificed every year in Europe in order to demonstrate freedom of a disease by lethal sampling as the only accepted method. Challenges in the development of serological techniques as IFAT, ELISA and sero neutralization in fish sera is described.

Diseases included are VHS, IHN, IPN, ISA and KHV. Kinetics of the antibody response, the lack of secondary immune response, and a surprisingly high specificity of antibody reaction giving high requirements to the quality of the antigens used in tests will be described. Antibody response is very dependent on water temperature where almost no antibodies can be detected at temperatures below 5°C before 3 months after infection while specific antibodies already are detectable in trout 2 wks after infection at 15°C.

Due to the strong dependence of correct folded epitopes in 3-D structures for recognition by fish antibodies live untreated virus have been used in the tests in order to preserve antigenicity. Procedures for gentle virus inactivation in order to reduce risk of contamination have been assessed and implemented.

Development and validation of serological techniques is an important task for WP 6.1 in EPIZONE. The achievements in the recent 4½ years have resulted in a number of proficiency tests, new developments and publications and a review on use of serology in aquaculture is in preparation.

ES 38: THE SUSCEPTIBILITY OF TURBOT PSETTA MAXIMA (LINNAEUS, 1758) AND BLACK SEA SALMON, SALMO LABRAX (PALLAS, 1814), TO GENOTYPE 1E STRAINS OF VIRAL HAEMORRHAGIC SEPTICEMIA VIRUS

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Key Words: viral haemorrhagic septicemia virus, susceptibility, turbot, Black Sea salmon, mutation

The susceptibility of Black Sea salmon, *Salmo labrax* and turbot, *Psetta maxima*, to eight strains of viral hemorrhagic septicemia virus (VHSV), was tested. Juvenile turbot of approximately 25 g and Black Sea salmon approximately 8 g weight were subjected to challenge by immersion (i.m.) and intraperitoneal injection (i.p.) to infection with the VHSV isolates representing the genogroup 1e. Infection trials showed that seven isolates were highly pathogenic for turbot by both i.p. and i.m. with mortality rates of 44-96% and 12-60% percent while one of the isolate was found not pathogenic on turbot by both challenges. All the isolates found none or low pathogenic on Black Sea salmon by both challenges. The results suggest that Black Sea salmon are less susceptible to VHSV genogroup 1e isolates, while turbot are highly susceptible. Some of unique mutations on immunological epitope motifs, which changed the amino acid substitution (aa 220 to 245), were determined on partial sequence of glycoprotein gene of a non-pathogenic isolate. As result; this could be a naturally attenuated strain and it has a potential to be vaccine candidate.

ES 39: THE VENETO REGION INFORMATION SYSTEM APPLIED TO THE MANAGEMENT OF EPIDEMIC EMERGENCIES: AN OVERVIEW

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IZS-Ve¹

Key words: information system, epidemiological surveillance networks, data

The recent onset or reappearance of serious re-emerging diseases in Europe (FMD, Bluetongue, West Nile Disease,) has highlighted the need for both a correct diagnosis and early detection of infected animals. Indeed, if an outbreak occurs in livestock, one of the first steps is to take effective and timely measures for disease control.

In order to ensure common standards among Member States, which is essential for free movement of animals across the European Union (EU), Directive 97/12/EC was adopted. In detail, it lays down the criteria for the establishment of an epidemiological surveillance network in each Member State consisting of the following actors:

- the herds,

- the owner or any other natural or legal person responsible for the holding,

- the approved veterinarian or the official veterinarian responsible for the holding,

- the official veterinary service of the Member State,

- the official veterinary diagnostic laboratories or any other laboratory approved by the Competent Authority,

- a computerized database.

With the purpose of ensuring rapid and accurate tracing of animals for animal health reasons, each Member State should create a computerized database recording all holdings on their territory, the identity of the animals and their movements. In Italy, Directive 97/12/EC was transposed into the Italian law by Legislative Decree n.196 of 22 May 1999. An information system (RIS) recording of data on both activities performed by official veterinarians on livestock and related holdings has been in place in the Veneto Region since 1998. The core of the RIS is the regional data bank of animal farms and related industries (RDB) that is linked to its national counterpart (National data bank – NDB) to guarantee the data flow required by the legislation in force. The RDB allowed the development of the RIS as a precondition for the management of sanitary programs and the surveillance activities performed by Veterinary Services (VS) in the Veneto Region. This feature has been applied as part of the automated exchange of information between IZILAB, the software which manages Official Laboratories activities, GESVET, the program used by the VS of the Local Public Health Units (LPHUs) and RDB archives. This system allows a greater availability of information in real-time and creates a network of experts on the management of possible emergencies and the selection of appropriate intervention measures in case of an outbreak. In addition, through the GESVET procedure, all the activities performed by the official veterinarians at the slaughterhouse are related to the surveillance activities conducted by LPHUs in the holdings of origin of slaughtered animals. Moreover the RIS is linked to a Geographical Information System (GIS) for spatial data management. A toolbox based on this GIS system is used to manage spatial data in case of an outbreak and it allows the automatic identification of the areas and holdings at risk so as to rapidly adopt appropriate field interventions.

ES 40: VARIABLES IDENTIFIED AS PREDICTORS OF CULEX PIPIENS DENSITY DURING THE 2010 ENTOMOLOGICAL SURVEILLANCE FOR WEST NILE DISEASE IN VENETO REGION, NORTH-EASTERN ITALY

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IZS-Ve¹; Regione Veneto²; Entostudio snc³

Key words: West Nile, Italy, entomological surveillance

In north-eastern Italy, following an outbreak of West Nile virus (WNV) infection involving humans, domestic animals and wild birds in 2008, a surveillance program was implemented based on serological, entomological and clinical monitoring. During 2010, the viral circulation in Veneto region was confirmed by clinical cases (6 humans and 4 horses) and by the WNV detection in 10 Culex pipiens pools. A high correlation among localities with viral circulation and Cx.pipiens density was noted. Therefore, trapping sites were ecologically characterized and a statistical analysis was performed aimed to find potential predictors of Cx.pipiens density.

Materials and Methods

Entomological monitoring was performed from May through November in 43 sites, covering the previously defined VCA (virus circulation area), SA (surveillance area) and on the bordering areas. CDC-CO2 traps were used for one night every 15 days. A buffer of 4 km surrounding each trap was considered and landscape data extracted (European Corine land cover 2006). Sites were then characterized as rural (areas devoted to agriculture), artificial (dominance of urbanized and industrial areas), semi-natural (forested and open semi-natural landscape) and humid areas (internal humid areas like ponds, marshes and lagoons) based on the dominant landscape type in each buffer. Others variables considered were altitude (range -2/221 mslm), presence of wild and domestic animals and disinfestation activity. Associations between variables and Cx.pipiens density were screened using the GLM (General linear model) procedure available in the SPSS software version 15.0 for Windows. Suspected interactions among variables were also included in the models.

Results

Overall, 137,965 mosquitoes of 16 species were collected, with a mean mosquito density/capture of 251.76. The majority of mosquitoes (93%) were represented by Cx.pipiens (87%), Ochlerotatus caspius (3.5%) and Aedes vexans (2.5%). The best predictors of Cx.pipiens density were: altitude (higher density below 60 mslm) followed by habitat type (i.e. rural and humid areas) and presence of horses (and not cattle or dogs and cats). There was a significant interaction between habitat and horses as effect on mosquito density. The presence of wild animals, both mammals and birds, and disinfestation activity seemed not to affect the mosquito density.

Conclusions

Cx.pipiens, the main vector of WNV in north-eastern Italy, is widespread in all the sites monitored. Apart from climatic variables, such as temperature and precipitation, which have been demonstrated so far to modulate the mosquitoes density and their pattern along the year, other variables can be considered as predictor of Cx.pipiens density. In the area monitored, rural and humid areas represent the best habitat, unlike other studies in USA that identified urban and periurban sites as the most suitable sites for this species. The data also suggest that horses may be more attractive for Cx.pipiens compared to cattle and pets, offering new hints for experimental studies. Although Cx.pipiens is normally considered ornithophilic, an association with wild or domestic birds presence was not found, however no data on density and species composition were available. Finally, the absence of evident effects of disinfestation on mosquito density stresses the importance of using standard methods to determine the efficacy of disinfestation.

Acknowledgement: This work was supported by the Public Health and Screening Service, Veneto Region and by the Italian Ministry of Health

ES 41: WEST NILE VIRUS IN EUROPE: A COMPARISON OF SURVEILLANCE SYSTEM TYPES AND SIZES IN A CHANGING EPIDEMIOLOGICAL CONTEXT

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Key words: West Nile virus, surveillance, Europe, optimization

In Europe, current knowledge suggests a low-level and recurrent circulation of West Nile virus (WNV), with occasional human and/or equines cases. However recent events lead to believe that this picture is changing and that Europe could experience a modification in the virus circulation patterns: (i) suspected endemic WNV circulation in Italy (Angelini et al. 2010) and Hungary (Krisztalovics et al. 2008); (ii) high diversity in WNV strains (at least four lineages) described in Europe for which pathogenic properties and public or animal health impact are poorly characterized (Bakoni et al. 2005; Kramer et al. 2008); (iii) Lineage II, which was described as mildly pathogenic for wild birds and equines but caused fatal neurologic equine cases in South Africa (Venter et al. 2009) and whose geographical distribution was supposed to be restricted to Africa and Madagascar, was detected for the first time in 2003 in Hungary (Bakoni et al. 2005) and spread afterwards throughout the country and in Austria (Calistri et al. 2010). In the light of these new epidemiological events, the risk that WNV emerges in Europe as a serious threat for horse and human health appears real but remains unpredictable. This uncertainty justifies the need of appropriate surveillance systems. We used a model of WNV circulation between Southern Europe and West Africa (Durand et al. 2010) to compute the sizes of equivalent West Nile surveillance systems, either passive (based upon horse populations and sentinel veterinarians) or active (sentinel horses or chickens WNV genome detection in trapped mosquito pools). For each surveillance system, minimal dimensions that ensured WNV detection (on a yearly basis) at a 95% confidence level were computed The costs and sensitivities of these surveillance systems were compared in three epidemiological contexts: a very low level circulation, a low level recurrent circulation, and an epidemic situation. For very low level situation and epidemic situation, a single parameter of the model was modified: the vector-host ratio of the European area. This ratio was increased (or decreased) until obtaining the desired yearly incidence rate in horses (62% or 1%). Passive surveillance on 1000 horses by specialized vet clinic appeared to be the most cost valuable and efficient system in the current European context, and estimated median dates of first detection appeared consistent with recent field observations. The proposed results could be used to adapt WN surveillance systems to local European epidemiological contexts.

ES 42: WILDPRO FOR WILDTECH – PUTTING WILDLIFE HEALTH DATA INTO CONTEXT

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Key words: WildPro, WildTech, Bluetongue, Tularaemia, Mycobacterium bovis

Wildpro® is an electronic encyclopaedia on the health and management of free-ranging and captive wild animals, and emerging infectious diseases. It provides detailed, fully referenced, peer reviewed information for professionals working in the fields of wildlife health and emerging infectious diseases. Each volume of the encyclopaedia is designed to integrate with other information from previously-completed volumes, as well as to operate on a stand-alone basis. The main encyclopaedia is enhanced by an extensive library of relevant full-text publications. The WildTech project aims to develop new methods to test for existing and emerging infectious diseases in wildlife species. As part of this project, data is being added to Wildpro on 30 infectious agents and diseases, many of which are important zoonoses while others are important in both wildlife and livestock. For WildTech, Wildpro will provide background information on the diseases , allowing the researchers to put their results into context in time, space and species affected. Detailed information is being added for five of these agents/diseases - Mycobacterium bovis (bovine TB), bluetonque virus (bluetonque), Francisella tularensis (tularaemia), hantaviruses (haemorrhagic fever with renal syndrome in humans) and European brown hare syndrome virus (EBHS). For the other agents/diseases, the emphasis will be on the European situation (species affected or infected, known distribution), and diagnosis. Wildpro's unique design makes it easy to find information and to see the links between different types of information. Wildpro will be fully Open Access from early in 2011.

Intervention strategies

IS 1: A GENERIC (-) RNA REPLICON VACCINE PLATFORM FOR CONTROL OF EPIZOOTIC DISEASES

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Key words: marker vaccine, replicon, safety, avian influenza, porcine reproductive and respiratory syndrome virus

Vaccines are one of the most effective preventive measures to control infectious diseases in livestock. Although classical vaccines types have been successfully employed in the past, they come along with several drawbacks. Certainly, whole inactivated and subunit vaccines are safe but they induce long-term and cellular immune responses less efficiently. To be effective, they require formulation with adjuvant, which may cause adverse effects, in particular if applied to mucosal surfaces. Live-attenuated vaccines are generally more powerful in inducing both humoral and cellular immune responses, which may last lifelong. However, there is a significant risk left that live-attenuated organisms eventually revert to virulence. It should be also noted that attenuation is a difficult and time-consuming process, which is not applicable to pathogens that cannot be propagated in vitro. With both inactivated and live-attenuated vaccines, serological differentiation of infected from vaccinated animals is difficult. The lack of efficacious marker vaccines is one argument, which led to the prohibition of general vaccination against epizootic diseases such as foot and mouth disease, classical swine fever, and avian influenza.

We are currently developing a novel safe and efficacious marker vaccine which is based on a deletion mutant of vesicular stomatitis virus (VSV) lacking the essential envelope (G) glycoprotein. Any gene encoding the antigen(s) of interest can be inserted into the VSVAG genome. The recombinant vector is propagated to high titers (109 infectious units/ml) on a VSV G protein expressing helper cell line. The complemented particles are infectious and mediate efficient delivery and cytosolic amplification of the cloned genes leading to high-level intracellular expression of the antigens and strong induction of both humoral and cellular immune responses. Importantly, infectious viral progeny cannot be generated due to the lack of the VSV G protein. This RNA replicon not only is non-pathogenic but also unable to revert to virulence. In addition, vaccinated animals will not produce neutralizing antibodies directed against the deleted G protein, thus allowing the use of VSV Δ G in prime/booster regimens. An important aspect is that young animals can be successfully vaccinated despite the presence of maternal antibodies. VSV Δ G vaccines will not interfere with standard VSV diagnosis based on VSV neutralization tests. VSVAG replicon vaccines do not require inactivation or formulation with adjuvant, and thus can be produced in a cost-effective way. The replicon particles are stable and can be stored lyophilized.

We will present data on the evaluation of this novel vaccine platform in chickens and pigs with respect to their effectiveness against avian influenza viruses and porcine reproductive and respiratory syndrome virus, respectively. We propose that RNA replicons provide the basis for a new generation of marker vaccines, which may be used in future surveillance and eradication programs.

IS 2: ASSESSMENT OF THE SAFETY OF BTVPUR AlSap® VACCINES IN PREGNANT EWES

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Key words: Bluetongue, sheep, vaccine, safety, pregnancy

Introduction

Following recent Bluetongue outbreaks in Europe, populations of domestic ruminants have been widely vaccinated against various serotypes of BTV. A very important proportion of these populations consist of pregnant female.

For ruminant producers, pregnancy of the female stock is the starting point of their production (meat / milk) and thus of most of their incomes.

It is therefore essential that the vaccines used are assessed and demonstrated safe in pregnant animals. We report here the results of a field study conducted to assess the safety of BTVPUR AlSap® vaccines in pregnant ewes.

From the results, it is concluded that administration of BTVPUR AlSap® vaccines to pregnant ewes is safe and does neither impact the reproductive performances of ewes nor the growth of their lambs.

Material and methods

To mimic any mono or bivalent combination of the BTVPUR AlSap® range, an overformulated bivalent BTV-2/BTV-4 vaccine was produced.

Four groups of fifteen ewes received a 1-mL subcutaneous injection of the vaccine under test or of a placebo, approximately at 7 or 18 weeks of pregnancy.

The animals were monitored as follows:

- daily clinical observation and recording of rectal temperature from the day of injection to the fourth day following injection;

- recording of the characteristics of the lambs at lambing;
- calculation of the body weight gain of the lambs at weaning;
- calculation of the total weight of weaned lamb per ewe;

Results

The vaccine did not induce any statistically significant increase of rectal temperature or abnormal clinical signs as compared to the injection of physiological saline.

No treatment-related impairment of the reproductive performance was reported since: - the number of born alive and dead lambs was not significantly different between groups,

- the number of lambs dead before weaning was not significantly different between groups,

- the relative average daily weight gain of lambs was not significantly different between groups,

- the total weight of weaned lamb per ewe was not significantly different between groups.

The safety of the vaccine tested was demonstrated in pregnant ewes during the first or second half of gestation through :

- absence of treatment-related general reactions or hyperthermia,
- absence of impairment of the reproductive performance of the ewes,
- absence of impairment of the growth of the lambs.

Conclusion

The safety of the vaccine tested was demonstrated in pregnant ewes during the first or second half of gestation through:

- absence of treatment-related general reactions or hyperthermia,
- absence of impairment of the reproductive performance of the ewes,
- absence of impairment of the growth of the lambs.

- Vaccines of the BTVPUR AlSap $\ensuremath{\mathbb{R}}$ range may safely be administered to pregnant ewes, throughout gestation.

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IS 3: CHARACTERIZATION OF AN E2-SUBSTITUTED C-STRAIN VACCINE CANDIDATE WITH POTENTIAL DIVA VACCINE PROPERTIES

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Key words: DIVA vaccine pestivirus CSFV

An advantage of the use of chimeric pestiviruses as modified live vaccines against classical swine fever (CSF) resides in their capacity to be manipulated to achieve the characteristics desired for safe and efficacious DIVA vaccines. We have recently described a new chimeric virus, Riems26 E2gif engineered specifically for this purpose (Rasmussen et al. 2010). The E2 substituted Riems26_E2gif was derived by homologues recombination of the full-length E2 protein encoding genome region from Border disease strain Gifhorn into a bacterial artificial chromosome (BAC) harbouring the complete genome of the CSFV vaccine strain C-Riems. This new chimeric pestivirus represents a C-strain based marker vaccine candidate. We have characterised the replication kinetics of Riems26_E2gif and compared it to the parental C-Riems clone. Autonomous replication of chimeric RNA could be observed after electroporation of in vitro transcribed RNAs into porcine PK15 cells. Further passage on PK15 cells revealed infectious chimeric virus with low titers. However, passage of the chimeric virus on ovine SFT-R cells revealed high titers of virus and were more efficient than passages on porcine cells. Data on characterisation of this new E2-substituted C-strain vaccine candidate will be presented and discussed in comparison to other chimeric viruses like CP7 E2alf and CP7 E2qif (Reimann et al., 2004; Rasmussen et al., 2007).

Acknowledgement: This study was supported by Danish Research Council for Technology and Production Sciences (grant 274-07-0198).

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IS 4: COMPARATIVE ANALYSIS OF THE PATHOGENESIS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS STRAINS

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Key words: PRRSV, pigs, pathogenesis, virulence, host response

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes respiratory disease and reproductive losses in pigs. It is extremely difficult to control worldwide by the appearance of new virus variants, and the insufficient protection by vaccination. In addition, highly virulent strains have emerged that led to high losses.

The objective of this study, part of the EU PoRRSCon Project, is to compare the pathogenesis of PRRSV strains in order to understand differences in virulence. More specifically, to define if there are differences in target cells that become infected and to dissect the immunological host responses of pigs infected with different strains.

Materials and methods

An animal trial was performed with 4 groups of sixteen pigs. In group 1, pigs were infected with EU type PRRSV strain Lena, known to induce clinical signs. In group 2, pigs were infected with the recently isolated EU type strain Belgium 07V063, that causes subclinical infections. In group 3, pigs were infected with the EU reference strain Lelystad (LV), that also causes subclinical infections. Group 4 were control pigs. At days 7 and 21 post inoculation (p.i.), 4 pigs per group were vaccinated with a pseudorabies (PR)vaccine to study the immune competence of pigs after PRRSV infection.

Weekly, serum was collected for antigen detection and antibody responses, and peripheral blood mononuclear cells were isolated for IFN-y ELISPOT assay and FACS analysis. With FACS analysis, kinetcs of the haematological changes were studied, focusing on the identification of the lymphocyte sub-populations.

At days 3 and 7 p.i., 4 pigs per group were euthanized for post-mortem examination. At day 35, 8 pigs per group (4 vaccinated and 4 non-vaccinated) were euthanized for post-mortem examination. Several tissues were collected for immunopathological analysis, to reveal PRRSV load and changes in leukocyte number and composition in tissues.

Results

Immunopathological analysis, PR serology, virus isolations and PCRs are currently performed and results will be presented during the conference. Preliminary data show that the animal trial resulted in the expected clinical pictures, with the Lena strain causing fever and respiratory symptoms, while the Belgium and LV strain caused subclinical infections.

All pigs inoculated with the Lena, Belgium or LV strain were infected and developed antibodies, detectable from 10 days p.i. Virus could be isolated in serum from the Lena and Belgium infected pigs between days 3-33 p.i, and from LV infected pigs between days 3-26. All control pigs remained uninfected.

FACS analysis showed differences in cell populations between the infected and control pigs (NK cells and $\gamma\delta$ T) and between strains (B cells and CD4 memory T).

The IFN-y ELISPOT assays showed an PRRSV specific IFN-y response at day 26 p.i., with the highest number of IFN-y secreting cells from Belgium infected pigs, followed by LV infected pigs and the lowest number by Lena infected pigs.

Discussion

The significance of the results will be discussed during the conference. The immunolopathological analysis will reveal if there are differences in PRRSV load between tissues and target cells that become infected. It is hypothesized that the more virulent Lena strain will infect more cell types of the monocytic cell lineage than the low virulent Belgium and LV strains. Based on the preliminary results, it can already been concluded that there are differences in pathological and immunological host responses between the Lena strain on one hand, and the Belgium and LV virus strain on the other hand.

IS 5: COMPARISON OF THE TRANSCRIPTIONAL RESPONSE IN BLOOD CELLS BETWEEN PIGS INFECTED WITH CLASSICAL SWINE FEVER VIRUS THAT DEVELOP A FATAL FORM OF INFECTION OR RECOVER

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Key words: Classical Swine Fever Virus, fatal form, recovery, transcriptional response, blood

Subtle differences in genetic background of viruses and host, as well as the health condition of the host, play a crucial role in how the host copes with a viral infection. In case of infection with moderately virulent strains of classical swine fever virus (CSFV) pigs either die from infection during the (sub)acute or chronic phase or recover after a (sub)acute phase. Pigs that die from infection excrete high levels of virus until death (high excreting form of infection), while pigs that recover excrete lower levels of virus (low excreting form of infection) (Weesendorp et al., 2009, 2010). To study the mechanism responsible for the development of high excreting pigs, fifteen pigs were inoculated with the moderately virulent Paderborn strain. By recording clinical signs of disease and determination of the excretion and transmission dynamics, we selected 5 pigs that developed the high excreting form, and 5 pigs that developed a low excreting form of infection. Whole blood of all pigs was collected in Paxgene-RNA tubes just before inoculation, and at day 4, 8,10, 12, 14, and 18 after inoculation. For each day of sampling an isogenic RNA pool (n=5) was prepared for both the high and low excreting group. Messenger RNA expression in each pool was measured using the pig Agilent 44K oligonucleotide array, and time dependent changes in gene expression were extracted from array datasets. On each day after inoculation markedly different changes in gene expression were observed between the high and low excreting group. Expression profiles showed that the activity of NK cells in the blood was enhanced in pigs with a low excreting form of infection and not in pigs with a high excreting form of infection. In the blood of pigs that developed a low excreting form of infection a characteristic change in gene regulation was observed around day 8-10 after inoculation, correlating with the onset of recovery. In contrast, in pigs with a high excreting form of infection regulation of specific clusters of genes was continued until the end of the trial (day 18). The significance of the host-processes specifically induced in the blood of pigs developing a high or low excreting form of infection will be discussed.

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IS 6: DEVELOPMENT OF ASFV EXPRESSING GREEN FLUORESCENT PROTEIN

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Key words: African swine fever virus, recombinant virus, EGFP

African swine fever virus (ASFV) is the agent of a highly contagious hemorrhagic disease of domestic swine, for which no vaccine has been so far obtained. It presents a major threat for animal production worldwide, particularly with the recent example of its introduction to Caucasian and neighbouring countries.

ASFV is a large double stranded DNA virus of approximately 170 kb, encoding about 150 major open reading frames. Strategies aiming at facilitating the study and manipulation of this complex virus are hence important. In this context, the generation of virus expressing fluorescent proteins as an easily identifiable selection marker would significantly increase the efficacy in generating virus mutants.

The low pathogenic ASFV field isolate NHV was used to generate a recombinant virus expressing enhanced green fluorescent protein (EGFP). For this purpose, a new cell line derived from wild boar, WSL, which proved to efficiently support NHV replication, was used as infection and transfection system.

An intermediate plasmid construct containing an EGFP expressing cassette driven by the promoter from VP72 major viral structural protein, flanked by thymidine kinase (TK) viral homologue gene left and right segments was developed to facilitate the selection of recombinant EGFP expressing virus (NHV_EGFP_TKminus) by infection of TK minus Vero cells in presence of BrdU.

The intermediate plasmid was initially transfected into WSL cells, followed by NHV infection. Emergence of fluorescent foci indicative of recombinant EGFP expressing virus was observed from 2 days post-infection. These foci were collected and added to TK minus Vero cell cultures in presence of BrdU. Vero TK-minus cells permitted replication only to a limited extent, however. Therefore NHV_EGFP_TKminus was recovered subsequently on WSL cells. Recombinant virus purity was confirmed by counter stain immunofluorescence for anti-P30 early viral protein expression and PCR amplification of the TK sequence. Thus, NHV_EGFP_TKminus allows immediate identification of infected cells, constituting a useful tool for further ASFV studies.

IS 7: DIFFERENTIAL EXPRESSION MODULATION FOR DEATH RECEPTORS AND LIGANDS INDUCED BY CSFV IN RELATION TO STRAIN VIRULENCE

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Anses¹

Key words: CSFV, death receptors, apoptosis, lymphocytes, virulence

Classical Swine Fever (CSF) is an immunodepressive disease characterized by an early depletion of lymphocytes count in blood that can be observed before the detection of the CSFV. The intensity and the kinetics of this cell depletion are depending on the virulence of the viral strain. The mechanism is not well described but it is attributed to the induction of an apoptosis process in lymphocytes. Furthermore, it has been suggested that the apoptotic pathway mediated by death receptors could be activated given the high levels of TNFa produced in macrophages, dendritic cells and serum isolated from pigs infected with highly virulent strains. Based on this hypothesis, the expression levels for genes corresponding to the 3 different death receptors pathways: TRAIL-DR4, FASL-FAS and TNFa-TNFR1 were investigated by RT-PCR in PBMC after CSFV infection, in relation to strain virulence. The receptor TNFR1, previously seen by microarray as no differentially modulated by CSFV infection, was not assessed by PCR.

Eight 7-weeks-old SPF pigs were oronasally infected with 106 TCID50 of either the highly virulent strain Eystrup or the moderately virulent strain Paderborn. Blood samples were collected before infection (D0) and Day 1 (D1), D2, D3 post-inoculation. PBMC were purified before Trizol RNA extraction. Real-time RT-PCR was performed using the one-step Brilliant II SYBR GREEN qRT-PCR master mix (Agilent Technologies Inc). The relative amounts of all assessed genes were calculated using the R = $2-\Delta\Delta$ CT equation.

The results for the TRAIL-DR4 pathway showed a significant up-regulation of the ligand TRAIL by both CSFV strains over the 3 days of infection. Up-regulation with Eystrup was obtained as early as D1 whereas with Paderborn, the relative expression level rose slowly to reach a ratio similar to that of Eystrup on D3. For the receptor DR4, the results showed an inverse regulation related to the CSFV strain with Eystrup displaying an immediate up-regulation on D1 whereas Paderborn showed a progressive down-regulation. The ligand of the FASL-FAS pathway was also inversely regulated i.e., up-regulated with Eystrup and down-regulated with Paderborn. A similar modulation profile to that of TRAIL was observed for the receptor FAS in spite of the modulation was much less pronounced for FAS than for TRAIL. Concerning the TNFa-TNFR1 pathway, the expression of the ligand TNFa was enhanced higher and faster with Eystrup whereas no significant modulation of TNFa expression was observed over the 3 days of infection with Paderborn. As TNFa may be involved in CSFV-induced apoptosis, the results obtained for TNFa at the RNA level were assessed and confirmed at the protein level by ELISA.

Our results suggest that both CSFV strains might sensitize PBMC to the death receptorsmediated apoptosis with correlation with the lymphocyte depletion induced. The delay observed with Paderborn for TRAIL and FAS up-regulations, the down-regulation of DR4 and FASL, as well as the weak induction of TNFa, might result in a diminished sensitivity of PBMC to death receptors-induced apoptosis, and might explain the delayed and reduced lymphopenia observed during Paderborn infection compared to that of Eystrup.

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IS 8: DNA VACCINATION OF TURBOT AGAINST VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS (VHSV)

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$\mathsf{VET}\text{-}\mathsf{DTU}^1$

Key words: turbot, DNA vaccine, glycoprotein, VHSV, antibody response

Turbot (Scophthalmus maximus) is a highly estimated fish species within aquaculture due to its delicious and tasty meet. As with other fish species, turbot occasionally suffers from infectious diseases when reared under intensive farming conditions. The negative stranded RNA-virus viral haemorrhagic septicaemia virus (VHSV) belonging to the family of novirhabdoviridae can cause problems for turbot at all life stages. The present studies were undertaken as a part of the Danish DAFINET collaboration to analyze the protective effect of a DNA-vaccine against VHS in turbot.

Turbot of approximately 5 g each were vaccinated by intramuscular injection with a DNAvaccine against VHSV. For comparison, a heterologous DNA-vaccine against IHNV, which is a related novirhabdovirus, was included. Eight weeks post vaccination, challenge was performed by immersion in a marine VHS-isolate or by ip-injection of a VHSV isolate from freshwater. The vhs-DNA vaccine protected the fish completely against VHSV independently of the challenge method, the mortality being lower by immersion compared to ip challenge. The ihn-DNA vaccine partly protected the fish against challenge, showing that part of the protection seen with the vhs-DNA vaccine was due to unspecific mechanisms. Blood samples taken 10 weeks post challenge showed high levels of neutralizing antibodies, in particular in fish given the vhs-DNA vaccine, suggesting that protection also was mediated by specific immune mechanisms, primed by the vaccine.

IS 9: EPIZONE: A HIGH-THROUGHPUT SCREENING AGAINST FOOT-AND-MOUTH DISEASE VIRUS OF A 35,000 SMALL CHEMICAL MOLECULES LIBRARY

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Key words: Foot-and-mouth disease virus, antiviral, in vitro, high-throughput screening

Introduction

Emergency vaccination is a legal tool to combat future outbreaks of foot-and-mouth disease (FMD) in Europe (European Council Directive 2003/85/EC). Limitations of the vaccination approach are the serotype-specificity of the vaccines and the delay of 7 days to reach complete clinical protection after vaccination (Golde et al., Vaccine, 2005). In order to minimize the need for animal culling during an FMD outbreak, additional alternative and complementary measures are required. Antiviral drugs could be used as an immediate, serotype non-specific control measure.

Aim

To develop an antiviral drug against FMD

Materials and methods

Approach 1: Five classes of compounds with reported in vitro activity against human picornaviruses (i.e. human entero- and rhinoviruses) were screened for their antiviral activity against FMD virus (FMDV). Five-hundred seventeen (517) molecules were each tested at 4 concentrations (i.e. 2-fold dilutions ranging from 50 μ M to 6.25 μ M) in an in vitro assay against FMDV strain O1 Manisa.

Approach 2: The chemical molecules library of the Center for Drug Design & Discovery (CD3) of the KULeuven contains 35,000 small molecules that were selected amongst others based on their drug-likeness. All these molecules were screened in vitro at a concentration of 25 μ M for a potential inhibitory effect on FMDV replication (strain O1 Manisa).

All in vitro antiviral assays were performed on SK-6 cells. Antiviral activity was assessed by light microscopic evaluation of cytopathic effect and by a colorimetric cell-viability assay that evaluates the activity of mitochondrial reductases and correlates with the presence of viable cells. Tetrazolium and resazurin were used in the first and second approach, respectively (Willems et al., J Virol Methods, conditionally accepted).

Results and future work

Approach 1: None of the 517 molecules exhibited substantial in vitro activity against FMDV.

Approach 2: One hundred and thirty-four (134) of the 35,000 molecules were identified as early hits (hit rate 0.38%). Anti-FMDV activity was confirmed in at least 2 independent tests for 27 out of the 134 early hit molecules. Based on the availability, the chemical properties and freedom to operate, 13 molecules from different molecular families were further selected. So far, we have evaluated 10 molecules; 5 of which efficiently reduced the RNA yield (100% reduction at 24h post inoculation (hpi) compared to untreated O1 Manisa-infected cells) and the infectious virus yield (2.1-5.0 log10 TCID50/ml reduction at 48 hpi compared to untreated O1 Manisa-infected cells) at concentrations that had no adverse effect on the host cells. A hit explosion will be initiated for the most potent inhibitors and analogues with superior activity and selectivity will be tested against the other 6 FMDV serotypes. Subsequently, the in vitro antiviral activity of at least 3 of these hit compound families will be further improved through a hit-to-lead optimization program, and their molecular mechanism of action will be studied. Following optimization, a preliminary in vivo assessment of the antiviral activity will be performed in an FMDV infection model in severe combined immunodeficient mice (Lefebvre et al., Transbound Emerg Dis, 2010).

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IS 10: EPIZONE: AN IN VITRO MODEL OF INTERACTION BETWEEN TYPE I INTERFERONS AND ORAL LYMPHOID TISSUES OF PIGS

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IZSLER^1

Key words: Tonsil, pig, cytokine, interferon, B lymphocytes

Low-dose interferon (IFN)-alpha treatments were shown to be effective in several models of infectious and autoimmune diseases. The clinical effects are generally induced at very low daily doses (0.1 - 1 IU / Kg bw), and disappear at higher ones, in line with established models of bell-shaped dose-response curves of cytokines both in vivo and in vitro. Early interactions of low-dose cytokines with oral lymphoid tissues are likely to exert crucial regulatory effects. These mainly affect inflammatory cytokine responses, rather than exerting direct antiviral or antibacterial effects. However, formal demonstrations of such a causal, cytokine / oral lymphoid tissue interaction are still lacking. This is the reason why we took to investigating in vitro the interaction of type I interferons (IFNs) and tonsil lymphocytes of pigs. These were obtained from 9 to 10-month old pigs at the slaughterhouse: after recovery in medium with a 5x antibiotic mixture (penicillin, streptomycin and amphotericin B) and overnight storage at + 4 °C, tissues were cut into 3- to 10-mm fragments with scissors, and lymphoid cells were pushed through a mesh using the flat end of a 30-ml plastic syringe plunger. Mononuclear cells were separated on Histopaque 1.077, washed, resuspended at 3 millions ml-1 and cultivated in medium with 1x antibiotics in 12-well Costar plates at 37 °C in 5% CO2 with different concentrations (0 / 1 / 100 IU ml-1) of human lymphoblastoid, porcine recombinant and porcine natural interferons alpha. Cell supernatants were harvested 18 hours later. Also, 0.2 ml of cells treated overnight with 1 IU /ml of interferons were transferred to untreated tonsil cells and further incubated for 24 hours under the same conditions.

Results can be summarized as follows:

- Tonsil lymphocytes treated with 100 IU/ml of IFNs significantly reduced residual bacterial

load after 18 hours in culture. This activity was traced back to antibacterial peptides released by cultured cells

- Release of IgA was effectively modulated by IFNs at both concentrations, and also by IFN-treated lymphocytes.

- Supernatants of IFN-treated cells caused significant reductions of spontaneous IL-8 release by swine intestinal epithelial cells (IPEC-J2 cell line).

Surface Ig-positive B lymphocytes were the prevailing cell population and their usage of surface Ig isotypes was shown to be very different from that of blood B lymphocytes. In this respect, a high frequency of IgA-secreting cells in tonsils were revealed by an ELISPOT assay. Our results indicate that tonsil cells can be activated by type I IFNs for crucial effector functions related to mucosal immunity and regulation of the inflammatory response also in distant sites. Therefore, the outlined model can be of some interest for further studies of low-dose oral cytokine treatments.

IS 11: EPIZONE: CHARACTERISTICS OF IN VITRO VACCINE POTENCY MODELS FOR FOOT-AND-MOUTH DISEASE

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VAR-CODA¹; Federal Centre for Animal Health (FGI ARRIAH)²; Intervet International BV³

Key words: foot-and-mouth disease, PD50 vaccine potency test, in vitro alternative, serology, serotype

Introduction

The in vivo 50% Protective Dose (PD50) test is the standard European procedure for foot-and-mouth disease (FMD) vaccine potency testing. Due to ethical, financial, logistic and bio-security reasons, current research alliances focus on the replacement of the challenge of cattle with virulent virus by serological in vitro alternatives. Another disadvantage of the in vivo test is its low repeatability and reproducibility (Goris et al., Vaccine, 2007). Previously, Goris et al. (Vaccine, 2008) validated a serological in vitro model for the FMD virus strain O1 Manisa. The present study aims at developing a comparable model for serotype A.

Materials and methods

Two groups of 85 cattle each were used for five replicates of in vivo vaccine potency tests. The first group was vaccinated with an A Iran 1996 (A96) vaccine in a homologous A96 challenge test. The second group was vaccinated with the same A96 vaccine, yet in a heterologous A22 Iraq 24/64 (A22) challenge setup. Serum samples were collected from all animals at 21 days post vaccination and their A96 and A22 antibody titres were determined in a Virus Neutralisation Test (VNT) and a Liquid Phase Blocking ELISA (LPBE) by two different laboratories, VAR and ARRIAH. The serological data were analysed with logistic regression and models for an alternative in vitro vaccine potency test were constructed as described by Goris et al. (Vaccine, 2008).

Results and Discussion

The homologous challenge of the cattle vaccinated with the A96 vaccine resulted in an in vivo PD50 of 20.5 with a 95% confidence interval (CI) between 13.1 and 23.5 PD50. For the serological in vitro alternatives, the PD50 varied between 5.9 (95% CI 5.2-10.8) and 13.1 PD50 (95% CI 11.4-19.9) for the VNT and the LPBE, respectively, and differences were observed between both laboratories. An indirect serological potency test may thus predict a lower PD50 than the in vivo test, but the variation of the in vitro tests was smaller than the variation of the in vivo test.

When the serological data were entered into the O1 Manisa potency model of Goris et al. (2008), the PD50 varied between 2.1 PD50 (95% CI 1.7-3.0) in the VNT and 13.1 PD50 (95% CI 11.3-19.9) in the LPBE. The even lower predicted PD50 in the VNT may suggest that serological in vitro alternatives predict the potency of highly potent FMD vaccines in a serotype-dependent manner.

The heterologous A22 challenge of the cattle vaccinated with the A96 vaccine resulted in an in vivo PD50 of 1.0 with a 95% CI of 0.8-1.6. When the heterologous A22 antibody titres were entered in the indirect PD50 models determined for the A96 vaccine and the O1 Manisa vaccine, the predicted PD50 ranged between 0.6 (95% CI 0.5-0.8 VNT) and 3.4 PD50 (95% CI 3.3-7.3 LPBE), and between 0.5 (95% CI 0.5-0.7 VNT) and 1.3 PD50 (95% CI 1.1-2.1 LPBE), respectively. This may suggest that serological in vitro models predict comparable low PD50 values as the in vivo test.

In conclusion, the present study suggests that serology-based alternative in vitro potency tests have to be validated for every individual laboratory and that their serotype-independency and vaccine potency robustness has to be investigated.

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IS 12: EPIZONE: COMPARATIVE ANALYSIS OF T CELL RESPONSES FOLLOWING EXPERIMENTAL INFECTION OF CALVES WITH BOVINE VIRAL DIARRHOEA VIRUSES-1 AND -3

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Key words: BVDV, T cell Immunity, Interferon-gamma, cross-reactivity, comparative dynamics

Bovine viral diarrhoea virus (BVDV) is an important cause of infectious disease of cattle that results in significant economic losses worldwide. BVDV exists in 2 recognized genotypes, BVDV-1 and 2. Additionally, a new atypical pestivirus genotype (BVDV-3) closely related to BVDV has recently been discovered. While there is some evidence of cross-protective responses of between BVDV-1 and BVDV-2 strains in spite of antigenic differences, there is no information on the antigenic cross-reactivity between BVDV-1 and BVDV-3. This work was conducted as part of an EPIZONE IC funded project, Comparative dynamics of BVDV, which involved experimental infection of calves with a BVDV-1 field isolate (Horton-916) and BVDV-3 (Khon-Kaen, Th/04), or a co-infection with both viruses. The aim of this study was to assess whether T cells induced by one virus are capable of responding to the heterologous virus and to identify targets of cross-reactive or distinct genotype-specific responses. The induction of T cell responses following experimental infection was assessed by ex vivo stimulation of peripheral blood mononuclear cells (PBMC) with the two viruses as well as with synthetic peptides representing the two major T cell antigens E2 (from the two viruses) and NS3 (from a 'reference' strain BVDV-1 Oregon C24V). IFN-y release as measured by ELISA was used as a read-out of T cell reactivity. Results showed that animals did not respond to both virus and E2 and NS3 peptide pools until 35 days post infection. There was limited cross reactive responses between the two genotypes. Animals from the co-infected group responded to both viruses and E2 peptides. PBMC were collected and cryopreserved longitudinally and work is underway to define antigenic regions and characterize the phenotype of responder T cells by multiparameteric flow cytometry. It is hoped that the data generated will have implications for the design of improved vaccines against BVD.

IS 13: EPIZONE: INTERLABORATORY RING TRIAL TO COMPARE DNA TRANSFECTION EFFICIENCIES

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Key words: DNA transfection, luciferase expression, interlaboratory ring trial

Chemical-based transfection of DNA into cultured cells is routinely used to study for example viral or cellular gene functions involved in virus replication, to analyse cellular defence mechanisms or develop specific strategies to interfere with virus replication. Other applications include rescue of viruses by reverse genetics and/or generation of mutated viruses. A large number of transfection chemicals like calcium phospate, branched organic compounds, liposomes, cationic polymers etc. are available on the market which are used by different laboratories for different cell lines. To obtain an overview on the efficiencies of varying transfection procedures, an interlaboratory ring trial was initiated within EPIZONE theme 5. A total of 15 participitating laboratories from 7 member institutions received RK13 cells, plasmid DNA encoding firefly luciferase under the transcriptional control of the human cytomegalovirus major immediate early promoter, a specially developed lysis buffer and a detailed protocol. Transfected cells were harvested in the laboratories of the participants, frozen and sent to the FLI where both the luciferase activity and protein content of the individual samples were determined to compare transfection efficiency between laboratories with the same protocol and equipment. In addition some laboratories sent samples from cells they are routinely using, transfected with the provided firefly luciferase plasmid, to allow comparison of transfection efficiency between different cell types. About 50 different samples were analysed and the luciferase activity per nanogram total protein (RLU/ng) was determined. The results revealed for RK13 cells a large range of specific luciferase activities between laboratories and, in comparison to RK13 cells, also varying transfection efficacies for other the cell lines. Details will be presented.

IS 14: EPIZONE: SEQUENTIAL DELETION OF GENES FROM AFRICAN SWINE FEVER VIRUS (ASFV) USING THE CRE/LOXP RECOMBINATION SYSTEM FOR THE PRODUCTION OF CANDIDATE ATTENUATED VACCINE STRAINS

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 IAH^1

Key words: ASFV recombinant virus vaccine

African Swine Fever Virus (ASFV) causes a severe haemorrhagic disease of domestic pigs. ASF is currently endemic in most sub-Saharan African countries, in Sardinia and in areas within the Trans-Caucasus region and Russian Federation to which it was introduced in 2007. Currently no vaccine is available to protect against ASFV.

The ASFV DNA genome is known to contain genes which encode proteins which are known to modulate the host immune system (Dixon et al 2004). In addition, we have determined the complete coding sequences of several high and low virulence ASFV isolates to identify genome changes that may be related to virulence. Deletion of specific ASFV genes responsible for immunomodulation and/or virulence may give rise to recombinant ASF viruses which act as successful vaccine strains.

Previously, recombinant ASF viruses have been constructed using a process of homologous recombination to delete single ASFV genes and insert a selectable marker gene. However a single marker gene can only be used once to generate a recombinant ASF virus. Using the cre/loxP bacteriophage system it has been shown in mammalian cells that specific genes can be deleted when cre recombinase is expressed (Schwenk et al 1995). Insertion of a selectable marker gene flanked by loxP sites into a recombinant ASF virus will allow subsequent removal of the marker gene and consequently the marker gene can be re-used for future ASF recombinant virus isolation.

Initial studies have used the ASFV tissue culture-adapted strain BA71V to create a recombinant virus in which the MGF genes 9L, 2R and 3R were deleted and the marker gene GUS was inserted. Subsequently, by transient expression of the cre recombinase, a second generation recombinant virus was isolated in which the GUS marker gene was deleted. A third generation recombinant virus was isolated following the deletion of the CD2v gene and re-introduction of the GUS marker gene. Having successfully demonstrated that the GUS marker gene could be inserted, deleted and reintroduced into strain BA71V in tissue culture, deletion of specific genes from the attenuated ASF virus strain OURT88/3 is now in progress.

IS 15: EPIZONE: SMALL REGULATORY RNAS OF THE RNA INTERFERENCE (RNAI) PATHWAY AS A PROPHYLACTIC TREATMENT AGAINST FISH PATHOGENIC VIRUSES

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Key words: RNA interference, microRNA, siRNA, prophylactic treatment

Small RNAs acting in the recently discovered gene regulatory mechanism called RNA interference has a potential as diagnostic signatures of disease and immunological state and when produced synthetically as prophylactic treatment of such diseases.

In the RNAi mechanism the cell produces different small RNAs which inhibit gene expression through more or less specific interaction with messenger RNAs resulting in repression of translation to protein. In this way cells can turn of genes of specific pathways thereby leading to altered physiological stages of tissues and possibly of whole organisms.

The mechanism can be programmed with several types of small double stranded RNAs - the type of which defines the destiny of the target. One such class of regulatory RNAs called microRNAs are upregulated due to various physiological responses of the cell and they suppress many genes simultaneously believed to be connected through common or related pathways. Another class of small RNAs, the so called small interfering RNAs (siRNAs) has received attention due their high degree of target specificity. Because synthetic siRNAs can be designed to target specific disease causing genes such as viral genes or oncogenes they hold promise in the treatment against cellular diseases in veterinary as well as human medicine.

This presentation will give an overview of the RNAi mechanism, and examples from our studies of microRNA regulation in rainbow trout during infection with the fish pathogenic rhabdovirus viral hemorrhagic septicemia virus (VHSV) and examples of some of our results on delivery and effect of siRNAs designed to target viral genes of VHSV. The VHS disease causes high mortalities in salmonid fish aquacultures why intervention strategies are highly in demand.

IS 16: GENE EXPRESSION PROFILING IN BOVINE MACROPHAGE CELL LINE (BOMAC) AFTER INFECTION WITH BOVINE RETROVIRUSES: BIV AND BFV

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$NVRI^1$

Key words: Microarray, BIV, BFV, Macrophage

Bovine Immunodeficiency Virus (BIV) and Bovine Foamy Virus are the members of Retroviruses family which are natural pathogens of cattle, highly prevalent worldwide. Both are viruses with no proven infectivity and its cellular trafficking still remains unknown.

To gain insight into host response to BIV and BFV infections we analysed the pattern of gene expression in bovine macrophage cells using BLO Plus microarrays from CAFG, Michigan State University. Bovine macrophage cell line (BoMac) was infected in vitro with BIV and BFV using supernatant harvested from FBL/BIV112 and Cf2Th/BFV100 infected cells respectively. Microarrays were hybridized using the two color hybridization method with total RNA extracted from BoMac and BoMac BIV or BFV infected cells at 72h post infection. Dye swap analysis was performed for each set of control and infected cultures. Subsequently Panther program was used to analyse biological functions and pathways of differentially expressed genes. Microarray comparison of BIV infected to uninfected BoMac cells revealed 62 upregulated and 92 downregulated genes. Pathways assigned for upregulated genes were related to angiogenesis, B cell activation, T cell activation, as well as endothelin, integrin and Wnt signaling pathways. Down regulated genes were involved in higher number of pathways including inflammation mediated chemokine and cytokine, integrin, PDGF and p53 signaling pathways. Analysis of microarray data for BFV infected BoMac cells revealed that 19 of genes were induced and 22 were suppressed at 72 hours after BFV infection. It was not possible to assign these genes for signaling pathways because for most of the genes biological and molecular function has not been classified. However biological process which were specifically induced after BFV infection included carbohydrate and protein metabolism, cell structure and motility and intracellular protein traffic. Surprisingly most of the down regulated genes corresponded also to carbohydrate and protein metabolism, intracellular protein traffic but also signal transduction and transport process. These findings will be confirmed by real-time PCR analysis and understanding of the mechanism of action of both viruses will be further examined.

IS 17: HEPATITIS E VIRUS AND INACTIVATION STRATEGIES

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CVI¹; VLA²

Key words: HEV; cells culture

Hepatitis E is responsible for over 50% of cases of acute viral hepatitis in non-industrialized countries associated with poor sanitary conditions. In developed countries the rate of mortality is between 1% and 4%. The virus is divided into 4 major genotypes but only a single serotype is recognized. Genotypes 1 and 2 are responsible for the major outbreaks in developing countries. Genotypes 3 and 4 are associated with sporadic cases in developed countries. It has been hypothesized that hepatitis E is a zoonotic disease and that there are animal reservoir(s) for HEV. The discovery of animal strains of HEV, as well as their demonstrated ability to infect across species provide strong support for this hypothesis. Zoonotic sources of food-borne transmissions of HEV from undercooked pig liver and deer meat to humans have been reported in Japan. It was shown that commercial pig livers purchased from local grocery stores as food in Japan, United States, and Europe are contaminated by HEV and that some of the HEV-contaminated commercial pig livers raises further public health concern.

The lack of an efficient and reliable cell culture system and a practical animal model for HEV have hindered studies on mechanisms of HEV replication, transmission, pathogenesis and environmental survival. Hence, an efficient in vitro propagation system for HEV is crucial for HEV research.

A 3D culture system, Rotating Wall Vessel, was optimized to grow HEV and subsequently HEV inactivation studies were performed. The object of this study was to test HEV inactivation through heating the inoculum (homogenate of infected pig liver) at different temperatures and by inoculations in PLC/PRF/5 cells.

RNA viral particles in the 3D cell culture were detected at all days post infection (dpi) in the cells infected with unheated inoculum. RNA viral particles were detected in the 3D system infected with inoculum heated at 56°C for one hour, at 0 dpi, 7 dpi and from 40 to 69 dpi. No viral particles were detected at any dpi in the 3D cells infected with inoculum heated at 100°C for 15 minutes.

These results confirm the findings of Feagin's et al (2007), showing that HEV can maintain its infectivity when heated at 56°C for 1hour. The results underline the potential of the 3D cell culture system of replacing traditional in vivo infectivity studies. An efficient cell culture system and reliable diagnostic procedures are useful tools to investigate the presence of HEV in the food chain and to assess the infectivity of the virus in food stuffs. The United States Department of Agriculture (USDA) and The United States National Pork Board (NPB) recommends a cooking method for fresh pork that will result in a minimum internal cooking temperature of 71°C.

Other strategies to inactivate HEV are on-going; such as UV light or detergents inactivation. The main aim of this experiment is to minimize the risk of human infection through contaminated pork by producing safety guidelines for pork consumers and workers in the pig food chain.

IS 18: HOST RESPONSE TO FOOT-AND MOUTH DISEASE INFECTION IN CATTLE; POSSIBLE IMPLICATIONS FOR THE DEVELOPMENT OF PERSISTENTLY INFECTED "CARRIERS"

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Key words: FMD, host response, pathogenesis

Foot-and-mouth disease (FMD) is a highly contagious viral disease with severe financial implications for countries dependent on substantial agricultural industries with export of related products. Any cloven hoofed animal species may become infected, and ruminants, especially cattle and buffalo, may develop into persistently infected "carriers" shedding low amounts of virus for several years after exposure to the disease.

Initial viral replication is believed to take place in pharyngeal epithelia, from where the virus spreads systemically causing characteristic vesicular lesions in areas covered by cornified epithelia. Mortality rates are low in adult animals but the morbidity is very high and the disease spreads rapidly amongst susceptible animals.

The host response to FMDV infection involves initial activation of the innate immune response, followed by subsequent production of high titres of anti-FMDV antibodies in the circulation. Antibodies are effective in clearing virus from the circulation, but in a proportion of animals (approximately 50 % in cattle) the virus is capable of persisting at a low level within pharyngeal tissue irrespective of the presence of neutralising antibodies in the circulation. The animals are defined as persistently infected (« carriers ») when live virus can be detected in pharyngeal excretions for more than 28 days post infection. The mechanisms involved in persistence of FMD in cattle are not fully known.

A series of animal experiments, with the aim of investigating the innate immune response and possible implications for the development of persistently infected FMD carrier-animals in cattle has been performed. Bull calves of 4-5 months of age were infected with FMDV O UKG 34/2001 and disease development was monitored for 35 days. Disease progression was monitored through observation of clinical signs and analysis of serum for the presence of viral genomes as well as FMDV-specific antibodies. Viral shedding was measured through qRT-PCR of mouth swabs and oropharyngeal fluid (probang samples).

Quantification of serum concentrations of acute phase proteins Serum Amyloid A (SAA) and Haptoglobulin (Hp), as well as biologically active type 1 interferon (IFN) indicated a clearly detectable acute phase response coinciding with the onset of clinical signs of disease. A novel experimental model allowing collection of small samples of pharyngeal epithelia and subjacent tissue from live animals by the use of an endoscope fitted with cutting biopsyforceps was developed. Biopsy samples from the dorsal soft palate were collected from each animal prior to infection and at three later timepoints during the course of the infection. Tissue samples were used for quantification of IFN a and $-\beta$, Tumor necrosis factor-a (TNF-a) and Toll-like receptor (TLR) 3 and -4 mRNAs plus FMDV genomes by qRT-PCRs.

IS 19: INFLUENCE OF AGE AND MATERNAL IMMUNITY ON THE ACTIVE POSTVACCINAL RESPONSE AGAINST INFLUENZA VIRUSES IN PIGS*

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$NVRI^1$

Key words: pigs, SIV, vaccination, maternal antibodies, humoral and cellular immunity

The influence of age and maternal antibodies (MDA) on the development and duration of postvaccinal response after vaccination of pigs with inactivated bivalent vaccine against influenza were investigated.

Fourteen sows and their litters were used. Seven from 14 sows were vaccinated (vacc.) 6 and 2 weeks before parturition. Piglets from sows vacc. (MDA-pos) and non-vacc. (MDA-neg) were divided into 6 groups. Two controls groups of piglets were non-vacc (MK (MDA-pos) and NK (MDA-neg)). Remaining groups were vacc. as follows: MB and NB at 1 and 4 weeks of life (wk), MC and NC at 1 and 8wk, MA and NA at 4 and 8wk, MD and ND at 8 and 10wk, ME and NE at 8 and 12wk.

To evaluate the T-cell response INF- γ secretion and lymphocyte proliferation were assayed. Antibodies to H1N1 and H3N2 viruses were determined using hemagglutination inhibition (HI) assay. All sera were tested in serial twofold dilutions, starting at 1:20. For estimates of the prevalence, titres equal or higher than 20 were considered positive. For statistical analyses titres lower than 20 were set to 5.

None of the non-vacc. MDA-negative pigs had antibodies against H1N1 and H3N2 viruses during the period of study, indicating, that no infection had occurred during the period of the experiment.

Neonatal MDA-pos piglets had high HI titres of antibodies against both viruses, reaching at least 2560 for H1N1 and 640 for H3N2 virus. MDA in the sera of piglets from group MK were above level considered positive until 13-14 wk for H1N1 and 9-10 wk for H3N2. The active humoral response against H1N1 virus in MDA-pos pigs were developed in animals from group MD and ME. In remaining groups the formation of HI antibodies was completely suppressed.

For H3N2 virus, seroconversion was noted in piglets from most vacc. groups, excluding these vacc. at 1 and 4wk (NB). Development of humoral postvaccinal response against H3N2 in most vacc. groups was probably the result of different kinetics of decline of antibodies against H1N1 and H3N2. The mean titre of MDA against H1N1 virus was over 60 up to 12 wk., while the mean titre against H3N2 decreased under 60 as early as 6 wk.

All MDA-neg pigs developed H1N1 and H3N2–specific humoral responses. The H1N1-specific response was the highest in groups NA, ND and NE. The H3N2-specific antibodies were also detected in all vacc. pigs. The levels of antibodies against H3H2 virus were similar in pigs vacc. at 1 and 4wk. and at 1 and 8wk. The highest HI titres in mentioned groups were about 100. In remaining vacc. groups the maximal HI titres were at least 240.

Piglets from group MB and NB, had the lower levels of antibodies than pigs that were older at the moment of vaccination.

There was no T-cell influenza-specific proliferation of PBMC in none of vaccinated groups. The values of SI after stimulation with both viruses in all vacc. pigs did not differ significantly from SI values in non-vacc. group. In vitro H1N1 and H3N2 stimulation did not induce production of INF- γ by PBMC in the case of either MDA-pos and MDA-neg immune or non-immune pigs. The results indicate that both, the age and MDA, influenced development of postvaccinal response.

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IS 20: NEW LIVE VACCINE AGAINST FOOT-AND-MOUTH DISEASE BASED ON RECOMBINANT CANINE ADENOVIRUS

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Key words: vaccine, canine adenovirus, foot-and-mouth-disease

To overcome limitations of conventional vaccines (containment facility and potential escape, non marker vaccine ...) alternative approaches are needed to control Foot-and-Mouth Disease (FMD). Adenovirus is a very promising delivery system for veterinary vaccines. Recombinant human adenoviruses expressing FMD virus (FMDV) antigens confer both cellular and humoral immunity. A single administration of a human adenovirus expressing capsid proteins of FMDV induces neutralizing antibodies and a rapid protection against a virulent challenge in swine and in cattle. Because veterinary vaccine based on human adenovirus is not suitable for mass vaccination, development of non human adenovirus expressing VP1 has been shown to induce humoral response; however the neutralizing antibody level was too low to perform a challenge trial.

The aim of the project is to evaluate a novel strategy to control FMD based on a marker vaccine which does not require high containment manufacturing facilities. This new vaccine derives from canine adenovirus type 2 (CAV2) which is used worldwide for the routine vaccination of dogs against serotypes 1 and 2 of canine adenovirus with an excellent safety record. The genome of FMDV encodes a polyprotein that is cleaved in structural and non structural proteins. The structural proteins (VP1 to VP4) are processed from a precursor (P1) by the 3C protease and form the virus capsid. We have developed vector from Cav2 that can accommodate insertion of large exogenous DNA such as P1 plus 3C coding sequences and express these FMDV antigens.

Evaluation of cross-reactive immunity against Cav vector in cattle and pigs

As a prerequisite, prevalence of a pre-existing immunity against CAV2 in sera from cattle and pigs has been assessed by neutralization assay using a CAV vector expressing GFP protein and a canine indicator cell line. However, since gene transfer using xenogenic AdV is efficient in pigs and cattle, irrespective of the immunological status against species-specific AdV, no or low cross-neutralization is expected from the latter assay. Results will be presented.

Production of Cav-FMDV vaccine

The expression cassette of the French 2001 FMDV serotype O strain P1 and 3C genes under the control of regulatory sequence from the IE gene of human cytomegalovirus (CMV) has been inserted into a shuttle plasmid designed for homologous recombination with CAV genome. Expression of the FMDV antigens will be presented following in vitro cellular transfection with CAV-FMDV plasmids using immunofluorescence and immunoblotting methods.

IS 21: NO FOOT-AND-MOUTH DISEASE VIRUS TRANSMISSION IN ONE WEEK VACCINATED CALVES

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CVI¹; Wageningen University²

Key words: foot-and-mouth disease, calves, transmission, vaccination

The aim of this study was to quantify the foot-and-mouth disease (FMD) transmission one week after vaccination in calves. Previous experiments showed that transmission was blocked two weeks after vaccination. , also most inoculated vaccinated animals resisted challenge. Studies in pigs, however, showed that vaccination 1 week before infection did not reduce transmission sufficiently. Transmission data in cattle one week after vaccination were lacking.

In total 46 calves of approximately 6 month of age were used. Direct transmission was studied in 10 pairs of calves either naïve or vaccinated one week before infection of one of the calves in each pair. Indirect transmission was studied by infecting 4 pairs of calves either naïve or vaccinated, After 3 days the inoculated calves were moved to a clean stable and the dirty stable was occupied by two naïve calves. The fifth group consisted of 2 calves as control of the vaccination response. Calves were infected with FMD Asia-1 virus by instillation in the nose with 2 times 1.5 ml virus suspension containing 6 log10 pfu/ml.Oropharyngeal swabs (OPF), urine, faeces and blood samples were analysed by virus isolation and titration. All OPF samples from the first 5 days were also analysed by RT-PCR. We recorded clinical signs, virus shedding and the development of antibodies. In addition, we determined whether the virus was transmitted from the inoculated calves to contact calves. In the naïve calves, all inoculated calves showed signs of FMD, fever, and vesicles on hoofs and/or in the mouth. Further, all inoculated calves shed virus in the different secretions and excretions, developed neutralizing antibodies and transmitted the virus to the contact exposed animals. The calves that were vaccinated one week before infection did not show any clinical sign, no virus could be isolated nor transmission was detected. This finding suggests that vaccination is capable of blocking transmission as soon as one week post vaccination, avoiding the spread of the disease.

IS 22: NOVEL STRATEGY FOR SEQUENCING AFRICAN SWINE FEVER VIRUS GENOMES

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FLI¹; FMV - UTL²

Key words: African swine fever virus, 454 sequencing

The recent 454 sequencing technology is providing rapid and important knowledge on full organism genomes. African swine fever virus (ASFV) is a large double stranded DNA virus with approximately 170 to 190 kbp genome, depending on the isolate. Recently, ASFV isolates of different virulence have been sequenced through this new technology, rapidly contributing to knowledge about this complex virus. One setback, however, is the need for a relative high amount of highly pure viral DNA, essentially free from contaminating cellular host DNA, which is sometimes difficult to obtain in a sufficient quantity for such a large scale-sequencing project.

With the aim of sequencing a highly and a low virulent homologous field isolate, L60 and NHV, respectively, we have developed an alternative approach. Both viral genomes were amplified by PCR with a high fidelity polymerase on a series of overlapping long amplicons of 10 to 22 kb, spanning the entire genomes. As template we used simply DNA extracted from in vitro infected natural host cells, swine macrophages, which is easily obtainable, obviating the need for highly purified viral DNA. Each amplicon was further submitted to electrophoresis and purified from agarose.

In a first attempt, a series of 10 kb amplicons covering approximately one third of the L60 and NHV genomes (kbp 50 to 110) were pooled in equimolar concentrations and sequenced by the 454 technology. This sequencing was successful and accurate, suggesting that in a next step, this approach should work also with longer/larger amplicon pools, namely covering the entire genomes in one single pool, making sequencing of any ASFV isolate easily attainable. Pools of 20 kbp amplicons are currently under study.

IS 23: ORIGINAL MODIFICATIONS OF PESTE DES PETITS RUMINANTS VIRUS (PPRV) GENOME INDUCED BY RNA INTERFERENCE (RNAI)

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CIRAD¹

Key words: Peste des petits ruminants (PPRV), RNA interference (RNAi), small interfering RNA (siRNA), escape mutant

Peste des petits ruminants virus (PPRV) is an enveloped non-segmented negative singlestranded RNA virus responsible for a contagious acute and fatal infection in domestic and wild small ruminants. The disease is widely spread in Africa, the Middle-East and South-West Asia. This virus is classified in the genus Morbillivirus within the family Paramyxoviridae. A live attenuated vaccine is available and provides a life-long immunity against PPRV, but no effective or specific treatments exist for infected animals. A new promising therapeutic tool to block the virus multiplication is the RNA interference (RNAi). RNAi is a process of sequencespecific post-transcriptional gene silencing that is triggered by double-stranded RNAs. The gene silencing by RNAi requires complementary with the target sequence and that single or few substitutions at the 5' and 3' ends are partially tolerated without loss of activity. A major problem of all antiviral therapies is the emergence of resistant variants. Many RNA viruses escape RNAi-mediated suppression by counteracting the RNAi machinery through mutations of the targeted region. The CIRAD previously identified three synthetic interfering RNA (siNPPRV1, siNPPRV6 and siNPPRV7) that target conserved regions of the essential gene encoding the viral nucleoprotein and prevent at least 90% of PPRV replication in vitro. In this study, we investigated the ability of PPRV to escape the inhibition conferred by these siRNA after several consecutive transfections in vitro with suboptimal siRNA concentrations. The mutations were characterized by sequencing and the relative quantification of the mutant and wild virus populations was obtained by QPCR. For the three siRNA, we recovered escape mutants with punctual nucleotide mutations (with or without amino acid substitution) or a deletion between 3 to 15 passages in cell cultures. Early appearance of escape mutants at each siRNA position was linked to the apparent lower degree of conservation of the target sequences within morbillivirus. For one of the mutants obtained, a deletion of six nucleotides in the target sequence of the siRNA was obtained shifting the open reading frame (ORF). However, this shift only resulted with a loss of 2 amino-acids, the rest of protein downstream being conserved. In addition to the conservation of the protein, it is also interesting to note that this deletion still fulfils the so-called 'rule of six' observed in morbillivirus, where only genome lengths multiples of six replicate efficiently. In contrast to single siRNA, a combination of two or three siRNA prevented the emergence of escape mutants after twenty passages in cell culture. This study provides new insights towards the genomic plasticity of morbilliviruses that should be considered in antiviral strategies conception.

IS 24: PORCINE INTERLEUKIN-2 SOLID LIPID NANOPARTICLES ENHANCES IMMUNE RESPONSE OF MICE TO FOOT-AND-MOUTH DISEASE VACCINE

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$LVRI^1$

Key words: Porcine Interleukin-2, nanoparticles, adjuvant effect

Introduction and Objectives

Vaccination is well documented to induce protective immunity to various infectious and noninfectious diseases in a range of animals, adjuvant have played a major role in successful vaccine development, such as cytokine, aluminum salts, monophosphoryl lipid A (MPL), QS-21 saponin and so on . Interleukin-2(IL-2) is a multifunction cytokine, which is vital to elicit the cellular and humoral immune response to foreign antigens. However, the utilization of IL-2 protein as adjuvant is still limited by its rapid degradation in vivo. In this study, we encapsuled the IL-2 protein into the solid lipid nanoparticles(SLN) to study the adjuvant effects.

Materials and Methods

pIL-2 was expressed in yeast expression system, and the purified recombinant IL-2 was encapsulated into nanoparticles with double emulsion solvent evaporation (W/O/W) method. To investigate the adjuvant effect, the BALB/c mice were intramuscularly co-injections with IL-2 Load nanoparticles and inactivated FMDV vaccine. The concentration of IL-4, IFN- γ was analyzed after second immunization 14d, and specific neutralizing antibody titer was detected by LBP-ELISA from immune mice blood after second immunization 0, 14d,28d,42d, and T cell proliferation analyzed in the vaccinated mice after second immunization 21d. The data analyzed by SPSS software.

Results

The results showed that the adjuvant of SLN- IL-2 initiated much stronger humoral immunity and cellular response . The titer of neutralizing antibody was up to 1:1200 in second immunization 14d, and significant higher than IL-2 protein +Ag+ blank nanoparticle and other groups (p<0.05) (fig1), In addition, the adjuvant of SLN-IL-2 induced significant(p<0.05) cytokines IFN- γ secretary and reached to 1083.47 pg/ml , and T-cell proliferation was up to 6.85% higher than other group (p<0.05) (fig2).

Conclusions

The results showed that SLN-IL-2 can delivery slowly in vivo and improved immune responses, which is a potential molecular adjuvant for enhancing both humoral and cellular responses to the foot-and-mouth disease vaccine.

IS 25: POSSIBLE ANTIVIRAL POTENTIAL OF SOLUBLE FORMS OF SIGLECS IN INFLUENZA VIRUS INFECTION

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Key words: Siglec-9, Siglec-5, influenza virus

Sialic acid-binding immunoglobulin-like lectins (Siglecs), a family of vertebrate endogenous receptors, are predominantly expressed by immune cells and recognize sialic acids on the cell surface. Sialic acids, the sugar residues located mostly at the outermost part of the glycans, are most suitable for recognition by endogenous lectins as well as by molecules of pathogenic origin. Influenza virus is one of the pathogens which utilize sialic acids as a viral receptor. Through interactions with a viral surface protein, hemagglutinin (HA), human influenza viruses bind preferentially to sialic acids containing N-acetylneuraminic acid a2,6-galactose (SAa2,6Gal) linkages while avian viruses bind preferentially to those containing N-acetylneuraminic acid a2,3-galactose (SAa2,3Gal) linkages. If sialic acids (the ligands of endogenous Siglecs) are also recognized by HA, this raises the possibility that Siglecs could competitively inhibit influenza virus from binding to its receptors on host cells. In the present study, we investigated the ability of Siglecs to inhibit influenza virus infection in vitro by using Madin-Derby canine kidney (MDCK) cells expressing a soluble form of human Siglec molecules.

MDCK cells were transfected with pCXN2 vector containing a chimeric gene expressing a fusion protein consisting of either the extracellular domain of human Siglec-9 or Siglec-5 fused to the Fc portion of human IgG2. Stable transformants (MDCK/Siglec9 and MDCK/Siglec5) were selected by their neomycin-resistant phenotype in the presence of 800 μ g/ml geneticin. Several clones of MDCK/Siglec9 and MDCK/Siglec5 were obtained. The expression of soluble forms of Siglec-9 (Sig9Ig) and Siglec-5 (Sig5Ig) in culture medium of individual clones was confirmed by Western blot analysis using anti-human IgG antibody. To measure the resistance of the MDCK cell clones expressing the soluble form of Siglec against influenza virus, a plaque formation assay was conducted. An H5N1 subtype highly pathogenic avian influenza (HPAI) virus (A/whooper swan/Aomori/1/2008), an H7N6 subtype low pathogenic avian influenza (LAPI) virus (A/quail/Aichi/1/2009) and an H8N4 subtype virus (A/turkey/Ontario/67) were used for plaque formation.

Among the MDCK/Siglec9 cell clones, clones 19 (Sig9-19) and 20 (Sig9-20) were chosen in order to evaluate the effects of relatively high level of Sig9Ig expression (Sig9-19) and low level of Sig9Ig expression (Sig9-20). Reduction rates of plaque formation in Sig9-19 to Sig9-20 were 88.8% for H5N1, 80.6% for H7N6 virus and 97.9% for H8N4 virus. As compared with the parent MDCK cells, the high expressing Sig9-19 showed a significant resistance, but not the low expressing Sig9-20. Other high expressing clones also reduced plaque formation. Similar results of plaque formation assay in MDCK/Siglec5 clones were obtained, although the reduction rates were lower (40 to 50%) than those of MDCK/Siglec9. These results suggest the possible antiviral ability of soluble forms of Siglec-9 and/or Siglec-5 against influenza virus infection in vitro.

IS 26: REGULATORY, RESEARCH & MANUFACTURING CHALLENGES FOR DEVELOPING FOOT- AND MOUTH DISEASE VACCINES FOR THE EU MARKET THAT PROTECT AGAINST NEWLY EMERGING FIELD STRAINS

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Key words: Foot-and-mouth disease strain adaptation

The Foot-and-Mouth Disease (FMD) Directive 2003/85/EC recognises the importance of vaccination as one pillar of the EU's animal health strategy to control incursions of the disease into the region. The Directive moves the potential of emergency vaccination to the forefront of disease control as an adjunct to other control strategies. Manufacturers, therefore, play a key role in the emergency supply of authorised vaccines of appropriate quality, safety and efficacy that contain the relevant vaccine strain(s) of FMD that match with the circulating field virus(es).

The recognition that the regulatory environment for veterinary vaccines in the EU is not always compatible with emergency supply of FMD vaccines containing new strains or formulations has led to a number of initiatives to facilitate the authorisation process. Adoption of legislation and an EU Guideline on multi-strain dossiers for diseases caused by highly-antigenically variable viruses should facilitate the availability of new FMD vaccines for the EU market in the event of disease outbreaks. The European Pharmacopoeia also provides for the rapid release of emergency vaccine prior to the results of all the final product tests under certain specific conditions. Storing FMD antigens at ultra-low temperatures in banks for rapid formulation has been a major breakthrough in this respect.

However, there is still much to be done to ensure the rapid availability of vaccines that protect against incursion of new field strains and that meet the appropriate regulatory requirements and are acceptable to veterinary authorities, the farming community and consumers of meat and dairy products.

The development and production of appropriate vaccines requires substantial investment, despite an uncertain market for the products, in the scientific, regulatory and technical issues to ensure the following conditions are met:

• They must be manufactured to EU principles of Quality Assurance and Good Manufacturing Practice as a minimum prerequisite.

• The use of the most appropriate virus strain(s) relies on an in-depth knowledge of the virus strains circulating in a region.

• New vaccine strain(s) must be adapted to cell-suspension culture, proven to be free of potential extraneous agents and shown to be safe and effective in the target animals which are indicated on the product literature.

• Starting materials must meet the requirements of the European Pharmacopoeia. In process and final product tests must be fully validated in accordance with GMP and Marketing Authorisation (MA) requirements.

• Vaccines must be shown to meet the minimum standard of the European Pharmacopoeia, which usually requires challenge studies of a large number of animals in high-containment facilities.

The challenges faced by industry in ensuring the availability of appropriate vaccines whilst meeting the necessary regulatory steps will be highlighted.

Risk assessment

RA 1: ASESSING THE IMPACT OF CLIMATE CHANGE ON MOSQUITOES WITH REGARD TO THE RISK OF INCURSION OF RIFT VALLEY FEVER VIRUS IN SOUTH-EAST ENGLAND

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Key words: climate change, rift valley fever virus, mosquito, risk

Rift Valley fever is a viral disease of animals and humans that occurs throughout sub-Saharan Africa, Egypt and the Arabian Peninsula. An expert opinion elicitation previously funded under Epizone WP 7.4 predicted the current risk of incursion of Rift Valley fever virus (RVFV) into the European Union as "negligible to low" but increasing to "low to medium" in the 2080s with climate change. The main routes were identified as through importation of livestock and mosquito vectors.

The work presented here describes the development of a geographical information system (GIS) approach to map the impact of climate change on the risk of incursion of RVFV in southeast England. The mosquito Aedes (Ochlerotatus) caspius has been chosen as the potential vector species for south-east England. Ae. caspius feeds mainly on cattle and sheep in England. Ae. caspius mosquitoes collected from southern France have been shown to be a competent vector for transmission of RVFV (Moutailler et al. 2008). Records of occurrence of Ae. caspius in England have been provided by the Health Protection Agency and mapped. For Ae. caspius, the egg is the overwintering stage and hatching occurs after flooding in the spring. Flood prediction data for extreme events have been obtained for south-east England from the UK Environment Agency. Central to the risk prediction is the recent publication of a mathematical model to predict the density of Ae. caspius from flooding using rainfall and temperature data (Balenghien et al. 2010). The UK Met Office have provided daily predictions for temperature and rainfall for 2078 to 2080. Major shipping ports and airports are located in the south-east of England. These are mapped and could serve as points of entry of tropical mosquitoes such as Ae. aegypti which could be infected with RVFV. Data are available on the origins of flights into the major airports. Cattle and sheep density data for south-east England have been mapped, and the approach identifies areas of greater risk for potential surveillance of livestock.

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POSTER: DAILY CULICOIDES SPP. (DIPTERA: CERATOPOGONIDAE) COUNTS IN VIENNA AND THEIR RELATION TO TEMPERATURE, PRECIPITATION, AND SOIL MOISTURE

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Key words: Culicoides, Bluetongue virus, monitoring, light trap, season

Culicoides spp. (Diptera: Ceratopogonidae) are the main vectors for the spread of viruses responsible for e.g. Bluetongue Disease (BTD), African Horse Sickness (AHS), and Rift Valley Fever (RVF). We establish a monitoring at the University of Veterinary Medicine Vienna, Austria, to determine species diversity, as well as the seasonal abundance of Culicoides spp. with two traps located outdoor and indoor a stable. Within the morphological determination it reveals that the dominant species observed during the investigation period 2009 was Culicoides obsoletus, 79.9% outdoor and 71.6% indoor. Amongst others also C. nubeculosus, C. pulcaris, and C. vexans have been identified, but no C. imicola or C. scoitus, the main vectors of BTD virus serotype 8 in Europe. To verify the morphological determinations a random sample of C. obsoletus and C. pulicaris was analysed with multiplex PCR. Prelimary results reveal that 86 % of the midges of the C. obsoletus complex and 94 % of the C. pulicaris complex were accurately identified. Relationships between environmental parameters and catches of midges were quantified by a statistical model based on cross-correlation maps (CCMs) and Poisson regression. We demonstrated that the seasonal cycle of Culicoides spp. abundance is well described by temperature, precipitation and soil moisture, respectively. Results comprise observed vs. modeled scale dependent correlation coefficients of r=0.74 (daily averages) up to r=0.95 (monthly averages) for outdoor counts of midges.

RA 3: DISTRIBUTION AND ABUNDANCE OF THE WILD BOAR (SUS SCROFA L.) IN THE IBERIAN PENINSULA BASED ON THE "CORINE" PROGRAM AND HUNTING STATISTICS

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Key words: wild boar, abundance & distribution map, iberian peninsula, corine, hunting

The wild boar is a key component of the European agro ecosystem, and its populations are increasing for years. The need for better knowledge of the wild boar populations has been enhanced recently due to the role of the species as a carrier of certain diseases which also affect domestic pigs and have a notable economic impact, such as African swine fever, classical swine fever or Aujeszky's disease. In Europe, and in the Iberian Peninsula in particular, changes in land use enable the expansion of the species, leading to an especially if its large population density and, consequently increase the probability of contact with farm animals and the transmission and maintenance of specific diseases. The objective of the present work is to yield an estimate of the size and distribution of the wild boar population in the Iberian Peninsula based on two main elements: hunting data and vital resources necessary for the wild boar to flourish.

When no data resulting from direct observations of wild boar are available in a long territory, yet hunting data can be obtained at the territorial level. A way to estimate the abundance and diversity of wild boars is to start from the numbers killed by hunters and then get to an approximation of the whole population by using a factor which represents the "hunting effectiveness". Thus, the total wild boar population in the Iberian Peninsula is estimated to be of 531699 (562975– 503715). In order to determine the potential habitat where the wild boar might thrive, various aspects of land use were selected (e.g. ability to supply sustenance and/or shelter to the animals), subjected to a thorough revision and given specific weights so as to lead, through a final spatial representation (ArcGIS 9.3. ESRI ®) in a choropletic map summarizing the suitability of various areas of the Iberian Peninsula for supporting wild boar populations.

Territory size and moving patterns for this species were used to arrive at a realistic habitat (Unified Habitat). The results obtained from adjusting the population estimates (animals killed in hunts x hunting effectiveness) to the potential habitat according to its importance were collated on a second map in order to estimate the density of wild boar per territorial unit in Portugal, in Spain and then in the Iberian Peninsula as a whole. Some discrepancies were observed between territorial limits sharing the same resources. The average density of wild boar in their potential habitat in Spain was 1.030/100 Ha with a range of between 0.039 and 6.176. In Portugal, the average density was 0.378/100Ha with a range of 0.001 to 5.532/100Ha, and in the Iberian Peninsula as a whole, 0.847/100Ha with a range of 0.001 to 6.176.

A second development is more accurate and incorporates an ecological regression using Bayesian hierarchical models, including factors or covariates and random components, which model the possible hidden factors to adjust the influence of the some factors used and soften the spatial location (neighborhood) of the data.

RA 4: LOW PATHOGENIC AVIAN INFLUENZA IN DOMESTIC POULTRY AND WILD BIRDS: EMPIRICAL APPROACH FOR A RISK BASED SURVEILLANCE

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Key words: Avian Influenza, Surveillance, Wild Birds

Several publications have encouraged the international standards as well as EU legislation to recommend risk based surveillance in each Member State. The study done in 2009 by Welby et al., (2010), in this context, led to the introduction of a new project (Flutree) aiming at refining the previous risk approach surveillance model, based on many assumptions regarding the major risk factors affecting the risk of infection and thus detection.

In this project, empirical data including wild bird surveillance and transmission experiments are used for proper estimates of the main parameters identified as affecting the sensitivity of the surveillance program. The project consists in 3 main work packages and is a collaboration between the ornithologists of the Royal Belgian Institute of Natural Sciences (RBINS), the virologists and the epidemiologists of Veterinary Agrochemical Research Center (VAR).

The aim of the presented study was to analyze the output of ongoing surveillance in wild birds and poultry holdings. Hereto, several analysis were carried out to measure the significance of the relative risk of contacts between wild birds and experimental poultry pens according to season, feed, proximity to wet land, and local abundance of wild waterfowl. Two poultry pens were selected for this study; one in urban area and another in more rural area. Within each poultry pen, continuous video recording was performed. A grand total of 21.780 h recording was obtained for the poultry pens under study. The observed variables were the number of incursions of wild waterfowl, the absence or presence of feed and/or water outdoor, whether the poultry pen is close wetlands or not, and the number of wild waterfowl count in the surroundings of the poultry pen, as well as the number of domestic birds within the poultry pens. A first step consisted in a descriptive analysis of the incursions over time, following which a time cluster analysis was performed to determine whether the cluster of incursions observed in time was significant or not. Non parametric and parametric separate univariate analyses were carried out to measure the significance of each risk factor separately before selecting the variables to be incorporated in a generalized linear mixed model.

The preliminary results enabled the identification of interesting features, amongst others, that characterization of risk zones based on wetland surface and higher migratory bird concentration might not be significant in increasing the risk of waterfowl contact with outdoor raised poultry, season seems to play a crucial role as well as the presence of feeding outdoor. This study has brought interesting results to light. Even though variability and uncertainty is distributed around these estimates, it will be important to take these results into consideration. In order to get the most reliable output from the scenario trees, it is important in these stochastic models to fit in accurate input parameters to enable proper inference. Integrating these parameters with the uncertainty and variability distribution around their estimation in the scenario tree will enable a better and thorough quantification of the sensitivity of the surveillance system.

RA 5: RISK ASSESSMENT OF EXOTIC VECTOR-BORNE LIVESTOCK DISEASES

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Key words: risk assessment, vector-borne diseases, framework

Increases in international trade and globalisation contribute to rapid and wide geographical spread of diseases. Furthermore, changes in climate, ecology, land use, and social welfare have contributed to the expansion of diseases beyond their endemic foci. The recent incursions of exotic vector-borne diseases into areas hitherto free from disease have urged the need for control of these diseases, both in the newly affected areas and in endemic regions. Better knowledge of these diseases and more insight into the possible pathways for introduction and subsequent spread is a prerequisite for efficient and (cost)effective risk management. Import risk analysis for livestock diseases is usually based on the guidelines given by the World Organisation for Animal Health (OIE). Assessment of the risk of introduction, establishment and spread of exotic vector-borne diseases requires, however, a multidisciplinary approach, taking into account epidemiology, virology, entomology, ecology and climatology.

A framework has been developed for risk assessment of exotic vector-borne livestock diseases that integrates the elements of various approaches for risk assessment that are currently used in disciplines such as animal health and pest risk analysis. The framework identifies the main steps in risk assessment of these diseases and provides a toolbox for (quick) assessments.

Five main steps are distinguished in the framework: (1) probability of entry, i.e., the probability that the pathogen causing the disease enters the area at risk by any route, (2) probability of establishment, i.e., the probability that the pathogen can spread to susceptible hosts implying that at least a competent vector should be present and that local environmental conditions are suitable for virus replication and spread, (3) extent of spread, i.e., the extent to which the pathogen is able to spread in time and space, considering both local dispersal and long-distance spread, (4) likelihood of persistence, i.e., the likelihood that the pathogen will assert itself in the area at risk for a prolonged period resulting in endemicity, and (5) impact of the disease being present in the area for the livestock sector and – if zoonotic – on human health, including economic, socio-ethical and environmental consequences. For each step, the framework gives (a) a flowchart identifying the key variables contributing to this step, (b) an extensive checklist with parameters that define the risk of this step, and (c) an overview of databases and methods available to qualify or quantify the risk of this step.

This framework provides risk analysts with a tool for risk assessment of exotic vector-borne livestock diseases, taking into account both likelihood of occurrence and potential impact. Results of these assessments will be used to inform stakeholders on behalf of their decision making. The primary stakeholders are governments and governmental bodies that need to decide on the risk management required to achieve the appropriate level of protection (ALOP) for the disease concerned. Risk assessments based on the framework will also provide insight into the main elements contributing to the risk which is a prerequisite when preparing for exotic vector-borne diseases. Furthermore, the framework will help to identify existing knowledge and data gaps that need to be solved to adequately address the risk.

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Key words: Zoonosis, Vector Borne disease, RVF, Risk Analysis

Rift Valley Fever (RVF) is a highly contagious viral vector-borne disease that affects both animals and humans. Particularly farm animals such as cows, sheep and goats are susceptible. RVF can cause abortion storms and high mortality rates in young animals. Infection in older animals may be non-specific or unapparent. Infection in humans is characterized by mild influenza-like symptoms. However, some people may experience more severe symptoms or complications. The most severe complication is a haemorrhagic fever which is often fatal.

RVF was first described in the Rift Valley of Kenya in 1930 and was confined to the African continent until 2000 when an outbreak of RVF was confirmed on the Arabian Peninsula. This outbreak led to significant mortality among farm animals and a high fatality rate of 14% in humans. RVF is now believed to be endemic in parts of the Arabian Peninsula.

Globalization and increased international trade as well as changes in climate and ecology are likely to have an impact on the geographical distribution of RVF. In the Netherlands several potential vector species for RVF are present. Introduction of the virus under favourable conditions might thus result in establishment of the disease in the Netherlands.

The aim of this study was to gain more insight into the possible pathways for introduction of RVF into the Netherlands and their relative risk. Results of the pathway analysis will help decision makers in prioritizing preventative measures.

A qualitative risk assessment was performed to estimate the likelihood of RVF introduction into the Netherlands via various routes, including international trade in live animals, animal products, goods, seasonal animal migration routes, and human travels. For each route a scenario tree was constructed to outline all steps required for successful introduction of RVF. Volumes of travel and trade were quantified according to region of origin. In the presentation, main results will be presented and discussed.





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